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Chemical profiling and quantitation of bioactive compounds in Platycladi Cacumen by UPLC-Q-TOF-MS/MS and UPLC-DAD

Bo Zhuang[a,1], Zhi-Ming Bi[a,1], Zi-Yuan Wang[a], Li Duan[b], Chang-Jiang-Sheng Lai[c], E-Hu Liu[a,*]

[a] State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University, No. 24 Tongjia Lane, Nanjing, 210009, China
[b] College of Chemistry and Material Science, Hebei Normal University, Shijiazhuang, 050024, Hebei, China
[c] National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, State Key Laboratory Breeding Base of Dao-di Herbs, Beijing, 100700, China

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ABSTRACT

Platycladi Cacumen (PC) is a traditional Chinese medicine used for the treatment of hemorrhages, cough, asthma and hair loss. To get a better understanding of the chemical constituents in PC, ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) and diagnostic ion filtering strategy were firstly employed for chemical profiling of PC. A total of 43 compounds including organic acids and derivatives, flavonoids as well as phenylpropanolds were unambiguously or reasonably identified. Coumarin and lignan were reported for the first time in PC. Chemical variation of 39 batches of PC from different geographical origins and 10 batches of processed product of PC was subsequently investigated by quantitation of nine major flavonoids. The results determined by UPLC coupled with diode array detection (UPLC-DAD) and hierarchical cluster analysis (HCA) indicated that the contents of flavonoids in PC samples differ greatly. This work provides an efficient approach to comprehensively evaluate the quality of PC.

1. Introduction

Platycladi Cacumen (PC), derived from the dried branches and leaves of *Platycladus orientalis* (L.) Franco, has been ubiquitously used in China for a long time. As a traditional Chinese medicine (TCM) and food additive, PC was recorded and summarized in ancient manuscript such as "Shen Nong Ben Cao Jing" and officially listed in the Chinese Pharmacopoeia [1]. PC has been applied in treatment of cough, gout, asthma, chronic bronchitis, hemorrhage disease and hair loss [1]. Recently, there has been growing evidence in different biological activities of PC, including antiphlogistic [2–4], antioxidant [5–7], hemostatic [8], neuroprotective [9,10] and hair growth promoting [11–13]. For example, the flavonoids in PC were reported to have a significant anti-inflammatory effect on lipo-polysaccharide-induced macrophage cells [2]. Also, essential oils extracted from different parts of PC demonstrated distinct antioxidant activity [7]. Cecarbon was confirmed to be a hemostatic compound by spectrum-effect relationship study [14]. Moreover, as a major constituent from PC, cedrol was considered as a hair growth promotor for its remarkable effects on hair growth and hair follicle length [11]. Platycladi Cacumen Carbonisatus (PCC), the legal processed product of PC, exhibits enhanced anti-hemorrhagic activity in pharmacological and clinical studies [14,15].

It has been well acknowledged that the efficacy of herbal medicines is significantly relevant to the chemical composition and the contents of active compounds in herbs. Compared with its long history of clinical use, chemical analysis and quality control studies on PC are rather limited. In previous literature, chemical analysis of PC was rarely performed for determination of one or a few of flavonoids, which are considered as the bioactive constituents responsible for the efficacy of PC [16–21]. There is still a lack of systematical research on the chemical profiling of PC. Also, the chemical variance of PC and PCC is seldom reported.

In the present study, both qualitative and quantitative analyses of chemical constituents were conducted for comprehensive quality control of PC. Ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) and diagnostic ion filtering strategy were employed for phytochemical profil-

* Corresponding author.
E-mail address: liuehu2011@163.com (E.-H. Liu).
[1] These authors contributed equally to this work.

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Table 1
Thirty-nine batches of PC.

Number	Origins	Number	Origins
PC01	Jiangsu	PC21	Henan
PC02	Jiangsu	PC22	Hubei
PC03	Zhejiang	PC23	Hubei
PC04	Anhui	PC24	Hunan
PC05	Anhui	PC25	Hunan
PC06	Anhui	PC26	Hunan
PC07	Jiangxi	PC27	Guangxi
PC08	Shandong	PC28	Guangxi
PC09	Shandong	PC29	Guangdong
PC10	Shandong	PC30	Guangdong
PC11	Shandong	PC31	Sichuan
PC12	Shandong	PC32	Guizhou
PC13	Shandong	PC33	Guizhou
PC14	Shanxi	PC34	Yunnan
PC15	Shanxi	PC35	Shanxi
PC16	Shanxi	PC36	Gansu
PC17	Hebei	PC37	Gansu
PC18	Hebei	PC38	Jilin
PC19	Hebei	PC39	Jilin
PC20	Henan		

ing of PC. Furthermore, 9 major flavonoids containing myricitrin, isoquercitrin, quercitrin, myricetin, afzelin, quercetin, kaempferol, amentoflavone and hinokiflavone in 39 batches of PC and 10 batches of PCC, were simultaneously quantitated using an UPLC coupled with diode array detection (UPLC-DAD) method. Hierarchical cluster analysis (HCA) was carried out to distinguish different batches of PC. Data presented in this investigation give a close insight into the phytochemical characterization of constituents in PC with the emphasis on flavonoids.

2. Experimental

2.1. Materials and reagents

A total of thirty nine batches of PC collected in present study were purchased from various Traditional Chinese Medicine market in eighteen province of China. The batch numbers were listed in Table 1. Ten batches of PCC were carbonized according to method of the Chinese Pharmacopoeia (2015 edition). Reference compounds of shikimic acid, citric acid, protocatechuic acid, procyanidin B2, rutin, epicatechin, *p*-coumaric acid, myricetrin, quercitrin, apigenin-7-*O*-*β*-D-glucoside, myricetin, quercetin, kaempferol, kaempferide, and amentoflavone were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and hinokiflavone was acquired from Meilune Biotech Co. Ltd (Dalian, China). Isoquercitrin was purchased from Must Bio-technology Co. Ltd (Chengdu, China). Kaempferol-3-*O*-rhamnoside (afzelin) was previously isolated from Ardisiae Japonica Herba extract in the authors' laboratory. The purity of all reference compounds were determined to be >98% by high performance liquid chromatography-diode array detection analysis. Deionized water used in experiments was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA). The LC/MS-grade acetonitrile was purchased from Merck (Darmstadt, Germany). The HPLC-grade formic acid was purchased from ROE Scientific Inc. (Newark, New Castle, USA). HPLC-grade acetonitrile was purchased from TEDIA (Fairfield, OH, USA). All other reagents and chemicals used were of analytical grade.

2.2. Preparation of standard solutions and sample solutions

Individual stock solutions of the references used for quantitative analysis were prepared by dissolving the each reference in methanol at a concentration of 1 mg/mL and then stored at 4 °C until use. A mixed standard solution was prepared by diluting stock solutions to desired concentrations with methanol.

The dry plant material was firstly ground into powder and sieved (60 mesh). For qualitative analysis, a total of 0.5 g plant material powder was accurately weighed, and then extracted by ultrasonicating (KQ5200B, 200W, 40 kHz, Kunshan, China) for 40 min with 20 mL 75% methanol at room temperature for each sample. For quantitative analysis, samples of 0.5 g of powdered PC and PCC were extracted with 10 mL of 75% ethyl alcohol by ultrasound-assisted extraction (UAE). The mixture was ultrasounded at room temperature for 60 min. All the prepared samples were followed by centrifugation at 16200 G for 10 min, and then analyzed directly. Each extraction was performed by triplicate.

2.3. Instrumentation and chromatographic conditions

2.3.1. Chemical profiling by UPLC-Q-TOF-MS/MS

Chromatographic analysis was performed on an Agilent 1290 Series HPLC system equipped with a diode array detector, a quaternary solvent delivery system and a column temperature controller. All the samples were carried out at a column temperature of 35 °C on a Thermo BDS Hypersil C18 column (250 mm × 4.6 mm, 5 μm, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of water with 0.2% formic acid (eluent A) and acetonitrile (eluent B) using a gradient elution mode of 10% B at 0–4 min, 10%–20% B at 4–24 min, 20% B at 24–30 min, 20%–23% B at 30–32 min, 23%–35% B at 32–44 min, 35%–50% B at 44–48 min, 50% B at 48–52 min, 50%–56% B at 52–55 min, 56%–95% B at 55–57 min, 95%–100% B at 57–60 min, 100% B at 60–65 min. The flow rate was 0.8 mL/min and the injection volume was 5 μL.

An Agilent 6530 QTOF tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was applied to MS and MS/MS detection. The operation conditions were as follows: drying gas (N_2) flow rate, 10 L/min; drying gas temperature, 350 °C; nebulizer, 35 psig; sheath gas flow rate, 11 L/min; sheath gas temperature, 350 °C; capillary voltages, 4000 V; fragmentor, 135 V; skimmer, 65 V; OCT RF Vpp, 750 V. The data were acquired in negative ion mode; collision energy was 15 V, 25 V and 35 V. Mass spectra were recorded across the range of *m*/*z* 50–1500. Agilent MassHunter Workstation Acquisition Software Version B.05.01 and Qualitative Analysis Software Version B.07.00 were utilized for system control, data acquisition, and data processing.

2.3.2. Quantitative analysis of nine flavonoids constituents in PC and PCC

Quantitative analysis was carried out on an Agilent 1290 Series HPLC system equipped with a diode array detector, a quaternary solvent delivery system and a column temperature controller. Chromatographic separation was conducted on a Thermo BDS Hypersil C18 column (100 mm × 4.6 mm, 2.4 μm, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase was composed with solvent A (0.2% aqueous formic acid) and solvent B (acetonitrile) with a gradient elution program: 0–3 min, 17% B; 3–6 min, 17–19% B; 6–7 min, 19–23% B, 7–11 min, 23–35% B; 11–13 min, 35–40% B; 13–14 min, 40% B; 14–16 min, 40–47% B; 16–19 min, 47–50% B; 19–20 min, 50–100% B; 20–24 min, 100% B. The constant flow rate was 0.8 mL/min and the column was maintained at 35 °C. The injection volume was 5 μL and the detection wavelength was set at 254 nm at 0–14 min and 340 nm at 14–24 min.

2.4. Data analysis

HCA was carried out based on the peak area of quantification of nine flavonoids compounds in all samples using SPSS Statistics 22 (IBM, Chicago, IL, USA) Software. When the target compounds were not detected or the contents of them less than the LOD in