Supplemental material

Identification and quantification of main flavonoids in the leaves of *Bambusa multiplex* cv. *Fernleaf*

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ABSTRACT

The chemical profile of *Bambusa multiplex cv. Fernleaf* (*B. multiplex*) leaves was analysed by UPLC-DAD-Q-TOF-MS. Twelve compounds were identified and *C*-glycosyl flavonoids, including vitexin, isovitexin, isoorientin and its derivatives, are the main constitutes of the plant. Besides, a HPLC method for isoorientin quantification was developed. The RSD of retention time and peak area were 0.05% and 2.04% for six times analysis of isoorientin with concentration of 20 μg/mL. The recovery of isoorientin in real sample was 99.2%. The general trend of isoorientin content in *B. multiplex* leaves was that it steady increased from Jan. to May, and then quickly decreased. The maximum was found on May with value of 4.7 mg/g. The lowest level of isoorientin was found during Aug. to Nov. with value of about 1.66 mg/g. In different seasons, isoorientin is always the dominant flavonoid which was accounted for about 50% of total flavonoids in the sample.

KEYWORDS: *Bambusa multiplex* cv. *Fernleaf*; *C*-glycosyl flavonoids; isoorientin; determination

Experimental

Plant material

Leaves of *B. multiplex* were collected in Jiangxi Agricultural University (with east longitude of 115°50' and northern latitude of 28°46') on different month during the year. The leaves were dried at 60 °C in oven. The dry material was smashed by high speed pulverizer and then filtered through 40 mesh sieve. The plant material was authenticated by Prof. Qing-Pei Yang (Jiangxi Agricultural University), and the

voucher specimen was deposited in Jiangxi Key Laboratory of Natural Product and Functional Food with number of JXNPF-122.

Chemicals

Isoorientin (>98%) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). HPLC grade acetonitrile was purchased Anhui Tedia High Purity Solvents Co., Ltd (Anqin, China). Milli-Q water was used throughout the study. All other reagents used were analytical grade.

Sample extraction

A aliquot of 0.1 g *B. multiplex* sample was mixed with 5.0 mL of 50% ethanol. After sonicating for 45 min in a bath sonicator (100 W, 45 kHz, Kunshan, China), the mixture was centrifuged at 4000 rpm for 5 min. The supernatant was filtered by 0.22 mm pore size filter and then used for HPLC and UHPLC-DAD-Q-TOF-MS analysis.

UPLC-Q-TOF-MS analysis

The chemical identification was performed on a Q-TOF 5600-plus mass spectrometer equipped with Turbo V sources and a Turbolonspray interface (AB Sciex Corporation, Foster City, CA, USA) coupled to a Shimadzu LC-30A UPLC-DAD system (Shimadzu Corporation, Kyoto, Japan). Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters）was used. The flow rate was 0.3 mL/min with injection volume of 1 μL and column temperature of 40 °C. The mobile phase was acetonitrile (A) and 0.1% formic acid aqueous solution (B) using a linear gradient program of 0-30min, 5-40% (A). The mass spectrometer was operated in the negative ion mode. Ultrapure nitrogen was used as the ion source gas 1 (50 psi), ion source gas 2 (50 psi), and curtain gas (40 psi). The Turbo Ion Spray voltage and temperature were set at -4500 V and 500 °C, respectively. Declustering potential, collision energy, and collision energy spread were set at 100 V, -40 V, and 10 V, respectively. Data acquisition was performed with Analyst 1.6 software (AB Sciex).

HPLC quantification analysis

The HPLC analysis was performed on an Agilent 1260 HPLC system equipped with an autosampler and DAD detector. A Symmetry C18 column (250 mm × 4.6 mm i.d., 5 μm; Waters, USA) was used as the stationary phase. The mobile phase consisted of

acetonitrile (A) and 0.1% acetic acid aqueous solution (B). The flow rate was 1 mL/min with linear gradient program of 0-30 min, 1-40% A; 30-35 min, 40% A. Detected wavelength was 349 nm with injection volume of 10 μL and column temperature of 40 °C.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) of triplicates. Statistical analysis, plotting, and curve fitting were performed by Origin 8.0 (Origin Lab Co., Northampton, MA, USA). One-way ANOVA was used for statistical analysis. Differences were considered significant when $P<0.05$.

Table S1 Mass characterizations of main peak in the chromatogram of *B. multiplex* by HPLC-Q-TOF-MS

Peak No.	RT (min)	$[M–H]^-$ (m/z)	Fragment ions (m/z) (% base peak)	Proposed structure	Reference
1	4.04	353.0875	191(100), 179(35), 135(70)	Caffeoylquinic acid	Weisz et al., 2009
2	5.41	355.0672	209(60), 191(50), 85(100)	*p*-Coumaroyl aldarate	Steingass et al., 2015
3	6.22	367.103	193(55), 134(100)	Feruloylquinic acid	Weisz et al., 2009
4	6.41	433.2072	179(70) , 89(100)	unidentified	
5	6.81	609.145	447(65), 357(98), 327(100), 298(20)	Isoorientin-7-*O*-glc	Ferreres et al., 2008
6	7.56	433.2073	387(100), 179(15), 119(30), 115(15), 89(38)	unidentified	
7	8.10	579.1348	489(50),399(80), 369(100)	Luteolin-6-C-arab-8-*C*-glc	Ferreres et al., 2008
8	8.57	609.1453	489(98), 429(55), 309(63), 327(62), 298(100)	Isoorientin-2"-*O*-glc	Ferreres et al., 2008
9	8.79	579.1357	459(80), 357(40), 309(55), 327(50), 298(100)	Isoorientin-2"-*O*-arab	Ferreres et al., 2008
10	9.08	447.0932	357(58), 327(100), 297(100), 285(60)	Isoorientin	Ferreres et al., 2008
		563.1403	443(45), 473(30), 383(65), 353(100)	Apigenin-6-*C*-arab-8-*C*-glc	Ferreres et al., 2008
11	9.98	563.1404	563(100), 401(30), 311(70), 297(35), 282(25)	unidentified	
12	10.29	563.1405	413(25), 311(10), 293(100)	Apigenin-*C*-hexoside-*O*-pentoside	Llorent-Martínez et al., 2015
13	10.64	431.0985	341(30),311(60), 283(100), 269(20)	vitexin	Li et al., 2006
14	11.26	431.0982	341(55), 311(100), 283(88), 269(15)	Isovitexin	Ferreres et al., 2008
		461.1083	371(25), 341(65), 298(100)	6-*C*-glycosylated flavonoid	Llorent-Martínez et al., 2015
15	12.99	723.5035	677(100)	unidentified	
16	13.28	563.2706	517(100), 317(98), 161(35)	unidentified	
17	14.07	836.5879	790(100)	unidentified	
18	14.94	949.6731	903(100)	unidentified	

Table S2 The content of isoorientin and total flavonoids in *B. multiplex* samples collected on different month

Collected data	Isoorientin（mg/g）	Total flavonoids[a]（mg/g）
2017.11.5	1.66±0.1[f]	3.53±0.34[e]
2018.1.4	2.61±0.04[d]	5.20±0.39[c]
2018.3.4	2.89±0.14[c]	5.55±0.45[c]
2018.4.5	3.49±0.09[b]	7.07±0.58[b]
2018.5.3	4.70±0.38[a]	9.17±0.54[a]
2018.5.28	2.97±0.12[c]	5.82±0.13[c]
2018.6.10	2.15±0.11[e]	3.84±0.15[e]
2018.7.18	2.31±0.13[e]	4.53±0.21[d]
2018.8.28	1.73±0.04[f]	3.79±0.05[e]
2018.9.27	1.75±0.06[f]	3.88±0.07e

[a]Calculated as isoorientin equivalent by substituting the area sum of peak 1 to 4 into the calibration curves of isoorientin. Different letter in column means significant difference (ANOVA, $p < 0.05$).

Figure S1

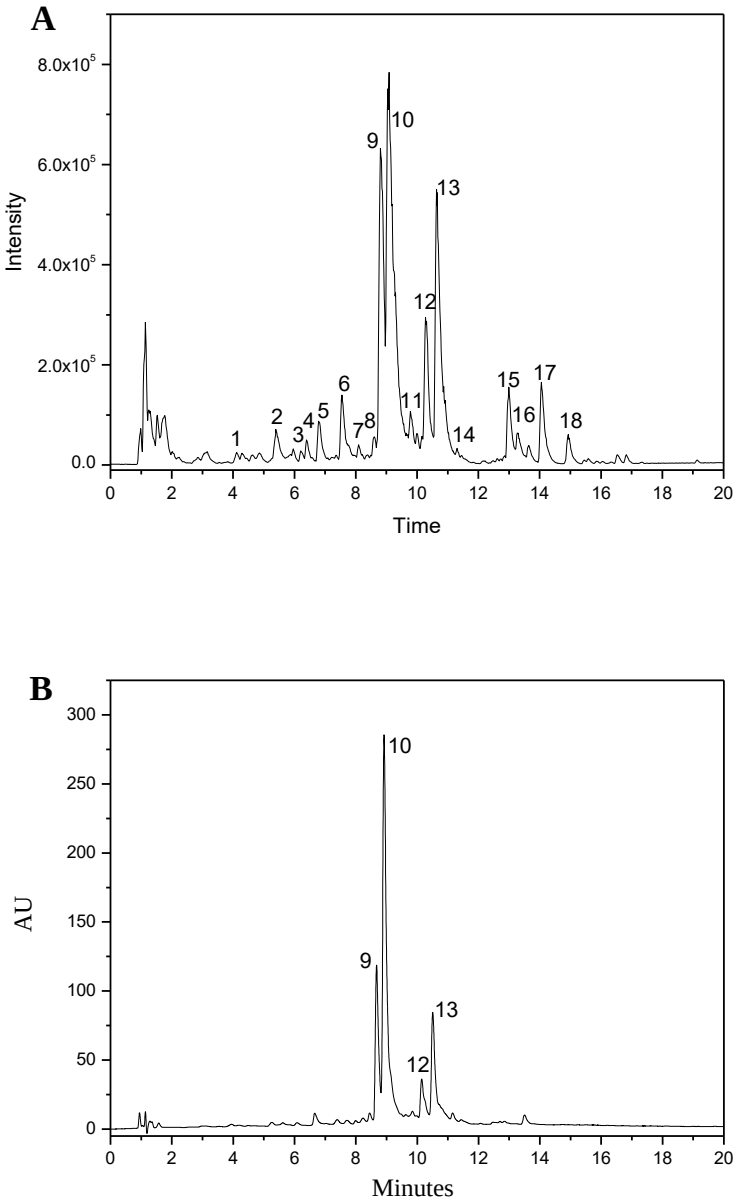

Figure S1 Base peak chromatogram (A) and absorbance chromatogram recorded at 349 nm (B) of *B. multiplex* extract analyzed by UHPLC-DAD-Q-TOF-MS.

Figure S2

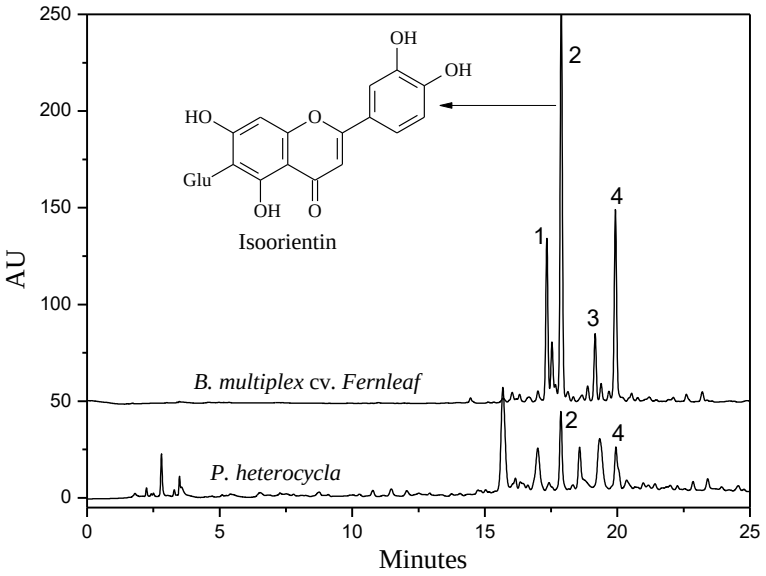

Figure S2. The HPLC chromatogram of *B. multiplex* and *P. heterocycla* (recorded at 349 nm), and the molecular structure of isoorientin. Peaks: 1, Isoorientin-2"-*O*-glc; 2, Isoorientin; 3, Apigenin-*C*-hexoside-*O*-pentoside; 4. vitexin

Figure S3

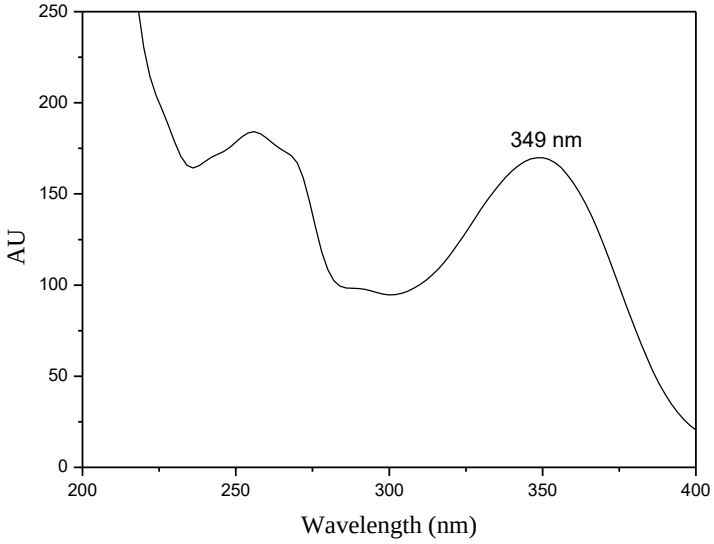

Figure S3 The UV spectra of isoorientin.

Figure S4

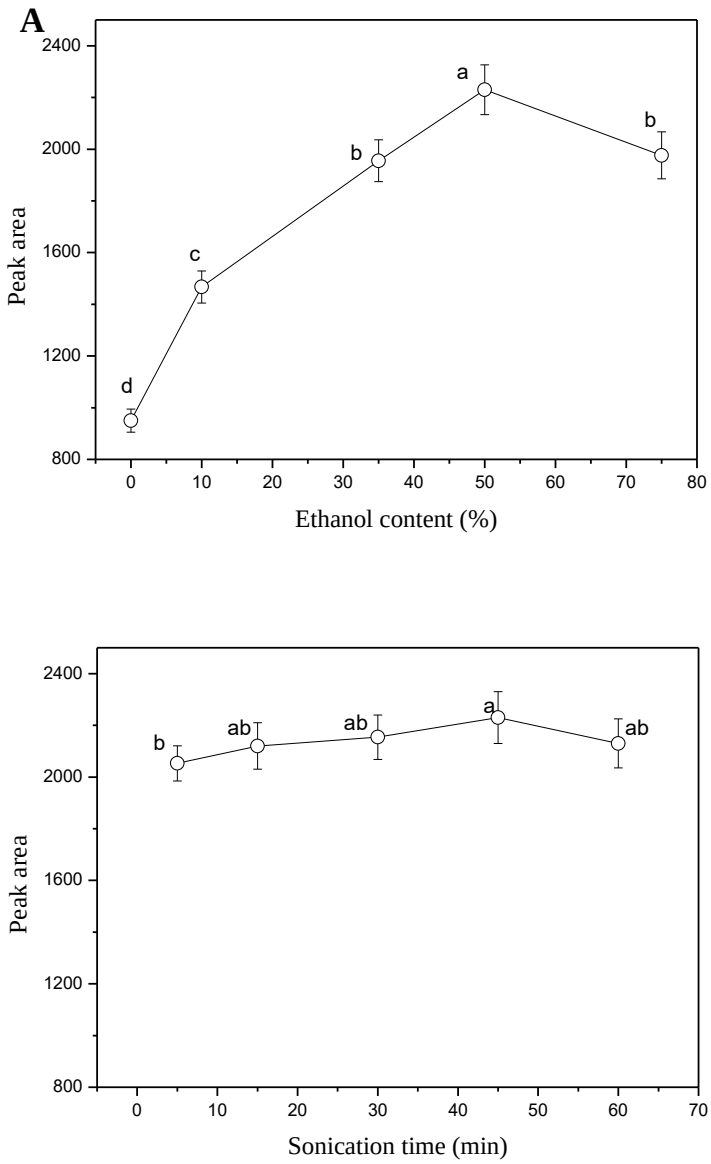

Figure S4 Isoorientin extraction optimization, effects of extraction solvent (A) and sonication time (B). Different letter in graph means significant difference (ANOVA, $p < 0.05$).

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