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Original Research Article

Simultaneous determination of ten compounds in two main medicinal plant parts of Tibetan herb, Pterocephalus hookeri (CB Clarke) Höeck, by ultra-high performance liquid chromatography-photodiode array

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Abstract

Purpose: *To develop an ultra-high performance liquid chromatography (UPLC) - photodiode array (PDA) method to compare the chemical composition of two different medicinal components of Pterocephalus hookeri.*
Methods: *Samples were chromatographically separated in succession using Waters Acquity UPLCR BEH C18 column (2.1 × 100 mm, 1.7 µm) and gradient elution (0.2 % phosphoric acid aqueous - acetonitrile). Using partial least squares discriminant analysis and one-way analysis of variance, attempts were made to distinguish different medicinal parts of P. hookeri.*
Results: *Regression equation for 10 compounds showed good linear regression (R^2 > 0.9994). The relative standard deviations of precision, stability, repeatability and recovery were under 5 %. Compared with the aerial plant part, the root had significantly higher levels of sylvestroside I (p < 0.01), cantleyoside (p < 0.001), dipsanosides B (p < 0.01) and dipsanosides A (p < 0.01), but significantly lower levels of loganic acid (p < 0.001), chlorogenic acid (p < 0.01), and isochlorogenic acid (p < 0.01). There were no significant differences between loganin, sweroside and isochlorogenic acid C.*
Conclusion: *The described method is simple, accurate and reproducible, and can be used for the simultaneous determination of 10 major compounds of P. hookeri. The results demonstrate that there is variation in the chemical composition of the aerialpart and root of P. hookeri and that loganic acid and cantleyoside are the primary chemical biomarkers.*

Keywords: *Tibetan medicine, Pterocephalus hookeri, Medicinal parts, Loganic acid and Cantleyoside, UPLC-PDA*

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INTRODUCTION

Pterocephali herba is the whole herb of the perennial plant *Pterocephalus hookeri* (C.B. Clarke) Höeck, a member of the Dipsacaceae family [1]. In Tibetan, *P. hookeri* is called “Bang-zi-du-wu,” and it is recorded as a Tibetan medical classic named “rGyud-bZhi” (usually known as the Four Tantras) [2,3] in “Drug Standards of Tibetan Medicines” [4] and the 2015 edition of the Chinese Pharmacopoeia [5]. As a traditional national medicine, *P. hookeri* has been widely used in many Tibetan herbal medicine formulations and is clinically used to treat diseases including common cold, rheumatoid arthritis, enteritis, dysentery, and other conditions [6,7].

Recent studies indicate that the main effective components of *P. hookeri* are glycosides, including iridoid glycosides (loganin and cantleyoside) and triterpenoid saponins (hookerosides A-D) [2,7,8]. Zhang *et al* [9] demonstrated that *P. hookeri* exhibit anti-inflammatory properties. To date, several analytical approaches including high-performance liquid chromatography (HPLC) [10], capillary zone electrophoresis [11], and ultra-fast liquid chromatography (UFLC) [12] have been used to quantify one compound or simultaneously quantify several compounds in *P. hookeri*. Partial least squares discriminant analysis (PLS-DA) is a supervised model based on dummy variable classification that can show the distribution and correlation between sample groups and has been used to distinguish between different species and identify potential biomarkers [13,14]. As far as we know, there is no publication describing the use of PLS-DA and simultaneous quantitation of 10 compounds to distinguish between different medicinal parts of *P. hookeri*.

Here we exploit a quick, dependable, and reproducible UPLC-PDA method for the simultaneous quantitative assay of 10 compounds in two different medicinal plant parts of *P. hookeri*.

EXPERIMENTAL

Materials and reagents

Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). Deionized water used for UPLC was obtained with the Milli-Q system from Millipore (Billerica, MA, USA). Other used reagents were of analytical grade.

The compounds (~98 %, quantification grade) of loganic acid **(1)**, chlorogenic acid **(2)**, and loganin **(3)** were obtained from the National Institutes for Food and Drug Control (Beijing, China). Sweroside **(4)**, sylvestroside I **(6)**, cantleyoside **(8)**, dipsanosides B **(9)**, dipsanosides A **(10)** were isolated and purified from *P. hookeri* by our research group [15-16]. Isochlorogenic acid A **(5)** and Isochlorogenic acid C **(7)** were provided by Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China).

A total of 32 batches of different plant parts of *P. hookeri* were collected from different regions of Sichuan and Yunnan Provinces in China, consisting of 16 batches each of aerial part and root samples. as listed in 'Results' section. The plant samples were authenticated by Professor Yi Zhang of College of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine, and voucher specimens kept in the herbarium of College of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine.

Instrument and chromatographic conditions

UPLC analysis was performed a Waters Acquity UPLC system (Waters Corp,. Milford, MA, USA), equipped with PDA. Acquity UPLC^{R} BEH C18 column (2.1×100 mm, 1.7 µm) was used. The mobile phase was made up of 0.2 % phosphoric acid aqueous (A) and acetonitrile (B). The gradient elution made was employed as follows: 0 - 4 min, 10 - 12 % B; 4 - 5 min, 12 - 17 % B; 5 - 10 min, 17 - 17 % B; 10 - 14 min, 17 - 25 % B; 14 - 15 min, 25 - 25 % B. Detection wavelength was monitored at 237 and 325 nm. Flow rate, 0.4 mL/min; injection volume, 1 µL; column temperature, 30 ^{o}C.

Preparation of standard solutions

The 10 compounds were precisely weighted and dissolved with 70 % methanol in a graduated flask to obtain mixed reference solutions of the following concentrations: 0.159 **(1)**, 0.305 **(2)**, 0.101 **(3)**, 0.175 **(4)**, 0.253 **(5)**, 0.385 **(6)**, 0.138 **(7)**, 1.385 **(8)**, 0.065 **(9)**, and 0.130 mg/mL **(10)**. The mixture solution was diluted stepwise with 70 % methyl alcohol to make standard solutions at different concentration ranges.

Sample preparation

All samples were smashed, and passed a 50 mesh sieve. Powdered samples (0.5 g) were accurately weighed and dissolved in 70 % methyl alcohol (50 mL) by sonication for 30 min. Additional 70 % methanol was added to make up for lost weight. The extract was filtered through a 0.22 µm microfiltration membrane.

UPLC analysis

Samples were analyzed with a Waters Acquity UPLC system. PDA was utilized for the detection using 237 and 325 nm. All chromatographic parameters were optimized for better separation, including mobile phase composition, flow rate, gradient elution mode, and column temperature.

The concentrations of loganic acid **(1)**, chlorogenic acid **(2)**, loganin **(3)**, sweroside **(4)**, isochlorogenic acid A **(5)**, sylvestroside I **(6)**, isochlorogenic acid C **(7)**, cantleyoside **(8)**, dipsanosides B **(9)**, and dipsanosides A **(10)** in every sample were calculated based on the established calibration curves.

Validation of UPLC method

The UPLC-PDA method was validated in the terms of linearity, stability, recovery, repeatability, and accuracy [17]. The storage solutions of 10 compounds were set up the regression equations, which were plotted after linear regression between the peak areas and concentrations of 10 compounds [18]. The limit of detection (LOD) and quantification (LOQ) were determined at signal-to- noise ratios of 3 and 10, respectively [19,20].

Data analysis

The UPLC-PDA data were input to the SIMCA-P software package (version 12.0, Umetrics, Umeå, Sweden) for PLS-DA [21]. GraphPad Prism software (version 5.0, GraphPad Software Inc, San Diego, CA, USA) was used to generate graphs and perform one-way analysis of variance (ANOVA) [22]. Differences were considered significant at $p < 0.05$.

RESULTS

Optimization of extraction procedure

To achieve an efficient extraction of the 10 compounds from *P. hookeri*, key factors including the method, solvent type, time, solvent volume, and number of extractions were independently investigated. A comparison of different extraction methods (ultrasonic, refluxing, and standing extraction) revealed no significant differences in extraction rate; however, ultrasonic extraction was most convenient. Methanol was a more efficient solvent than ethanol. The concentrations of three different aqueous methanol solvents (50, 70 or 90 %) were compared, and 70 % was chosen. Furthermore, tests of the number of extractions (1, 2 or 3 times), solvent volume (30, 50 or 70 mL), and time (20, 30 or 40 min) were investigated. The sample processing method was finally established as follows: 0.5 g sample was extracted with 50 mL of 70 % methanol with ultrasonication for 30 min.

Optimized UPLC conditions

Acquity UPLC^R BEH C18 (2.1 mm × 100 mm, 1.7 µm), Acquity UPLC^R BEH C18 (2.1 mm × 50 mm, 1.7 µm), and Acquity UPLC^R HSS T3 (2.1 mm × 150 mm, 1.8 µm) columns were put to test for their separation ability. The Acquity UPLC^R BEH C18 column (2.1 mm × 100 mm, 1.7 µm) was most effective. We also compared four different mobile phases (methanol-water, acetonitrile-water, acetonitrile-0.2 % formic acid aqueous solution, and acetonitrile-0.2 phosphoric acid aqueous solution) and found that acetonitrile-0.2 % phosphoric acid aqueous solution had lower column pressure, better peak shape, and higher resolution than other mobile phases [23]. Furthermore, the chromate-graphic conditions were tested incorporating the flow velocity (0.3, 0.4 or 0.45 mL/min) and column temperature (25, 30 or 35 °C), and separation was satisfactory for 30 °C and a flow rate of 0.4 mL/min. Based on the absorption curves of the tested analytes, loganic acid **(1)**, loganin **(3)**, sweroside **(4)**, sylvestroside I **(6)**, cantleyoside **(8)**, dipsanosides B **(9)**, and dipsanosides A **(10)** exhibited maximum absorptions at 237 nm, while chlorogenic acid **(2)**, isochlorogenic acid A **(5)**, and isochlorogenic acid C **(7)** showed maximum absorptions at 325 nm. Therefore, the PDA detection wavelengths were set at 237 and 325 nm. The UPLC-PDA results of 10 compounds are displayed in Figure 1.

UPLC validation data

The method was validated by the guidelines for Validation of Quality Standard of Traditional Chinese Medicine [24]. Table 1 lists detailed information. All regression equations showed good linear relationships ($R^2 > 0.9994$). LODs and LOQs for the 10 compounds were in the range of 0.12-3.35 µg/mL and 0.45-11.20 µg/mL, respectively. Table 2 provides detailed data on the precision, repeatability, and stability for 10 compounds, relative standard deviations (RSDs) were in the range of 0.44-0.96, 0.42-1.43, and 0.91-3.58, respectively. Table 3 shows the recovery rates for three various concentrations (low, middle, and high). The mean recovery rates were in the range of 96.62 to 100.76 %, and RSDs of the 10 compounds were less than 5 %. These findings demonstrate that the UPLC-PDA method is sufficiently precise and accurate for simultaneous quantification of 10 compounds in *P. hookeri*.

Method application to samples

Using the developed UPLC method, 10 compounds were determined simultaneously in 32 batches of different medicinal parts samples. Figure 1 shows representative the UPLC-PDA chromatograms of two different medicinal parts of *P. hookeri*. All samples were independently assayed three times to calculate the average content (mg/g), the results are shown in Table 4. Among the 10 compounds, dipsanosides A (0.30-9.52 mg/g) were more abundant than dipsanosides B (0.17-3.16 mg/g). In the root samples, the detected ranges were 1.60-9.13 mg/g for loganic acid, 0.87-16.55 mg/g for chlorogenic acid, 0.47-2.09 mg/g for loganin,

Table 1: Regression data, LOD and LOQ of 10 compounds

Compound	Calibration curve	R^2	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
Loganic acid (1)	y=2924.3 x – 2352.6	0.9994	2.48-158.75	0.51	1.75
Chlorogenic acid (2)	y=5949.3 x – 1234.8	1.0000	9.53-305.00	0.39	1.44
Loganin (3)	y=3082.1 x – 8.3	0.9999	3.15-100.63	0.30	1.12
Sweroside (4)	y=2489.9 x – 1515.3	1.0000	5.47-175.00	1.17	3.97
Isochlorogenic acid A (5)	y=6450.9 x – 33.1	0.9997	7.89-252.50	0.12	0.45
Sylvestroside I (6)	y=2956.1 x – 19.6	1.0000	3.01-385.00	0.58	1.93
Isochlorogenic acid C (7)	y=6679.9 x – 875.9	1.0000	4.30-137.50	0.33	1.18
Cantleyoside (8)	y=2019.0 x – 6031.3	0.9999	43.28-1385.00	3.35	11.20
Dipsanosides B (9)	y=2661.2 x – 1241.3	0.9999	2.03-65.00	0.69	2.29
Dipsanosides A (10)	y=3249.3 x – 1439.4	0.9998	2.03-130.00	0.70	2.37

Table 2: Precision, repeatability and stability of the UPLC method for determination of the 10 compounds

Compound	Precision (n=6)		Repeatability (n=6)		Stability (n=6)	
	Content (mg/g)	*RSD (%)*	*Content (mg/g)*	*RSD (%)*	*Content (mg/g)*	*RSD (%)*
Loganic acid (1)	3.46	0.53	3.47	0.60	3.44	1.26
Chlorogenic acid (2)	2.74	0.61	2.73	0.59	2.73	0.91
Loganin (3)	2.83	0.70	2.82	0.92	2.80	1.37
Sweroside (4)	5.44	0.55	5.44	0.46	5.43	2.23
Isochlorogenic acid A (5)	1.64	s0.77	1.63	1.15	1.66	1.01
Sylvestroside I (6)	26.44	0.75	26.49	0.89	26.57	0.95
Isochlorogenic acid C (7)	3.30	0.87	3.30	0.87	3.25	1.26
Cantleyoside (8)	56.76	0.44	56.76	0.42	56.83	0.59
Dipsanosides B (9)	1.58	0.96	1.57	1.43	1.55	3.58
Dipsanosides A (10)	2.56	0.68	2.56	1.10	2.53	1.08

Table 3: Recovery of the ten compounds

Compound	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
Loganic acid (1)	0.864	0.712	1.549	96.21	98.78	2.46
	0.864	0.855	1.728	101.05		
	0.865	1.094	1.949	99.09		
Chlorogenic acid (2)	0.683	0.55	1.247	102.55	99.65	2.53
	0.682	0.623	1.292	97.91		
	0.683	0.802	1.473	98.50		
Loganin (3)	0.705	0.648	1.386	102.31	97.73	1.48
	0.705	0.734	1.449	101.36		
	0.706	0.859	1.553	98.60		
Sweroside (4)	1.361	1.09	2.428	97.89	100.76	1.91
	1.36	1.415	2.762	99.08		
	1.362	1.608	2.909	96.21		
Isochlorogenic acid A (5)	0.409	0.306	0.701	95.32	99.36	3.55
	0.409	0.412	0.825	100.97		
	0.409	0.504	0.922	101.79		
Sylvestroside I (6)	6.554	5.332	11.785	98.11	98.86	1.79
	6.549	6.407	12.801	97.58		
	6.559	7.477	14.102	100.88		
Isochlorogenic acid C (7)	0.825	0.644	1.449	96.89	96.62	1.52
	0.825	0.805	1.59	95.03		
	0.826	0.966	1.772	97.93		
Cantleyoside (8)	14.095	11.593	25.485	98.25	99.02	1.04
	14.084	14.342	28.23	98.63		
	14.107	16.991	31.13	100.19		
Dipsanosides B (9)	0.392	0.294	0.673	95.58	98.73	2.87
	0.391	0.376	0.771	101.06		
	0.392	0.455	0.845	99.56		
Dipsanosides A (10)	0.642	0.543	1.173	97.79	98.53	1.18
	0.641	0.674	1.301	97.92		
	0.642	0.754	1.395	99.87		

2.04-5.10 mg/g for sweroside, 1.31-16.34 mg/g for isochlorogenic acid A, 0.51-10.86 mg/g for sylvestroside I, 1.34-9.79 mg/g for isochlorogenic acid C, 6.10-31.16 mg/g for cantleyoside, 0.17-0.74 mg/g for dipsanosides B, and 0.30-2.22 mg/g for dipsanosides A. In the root samples, cantleyoside always content the highest, ranging from 38.81 to 99.28mg/g. The contents were 0.26-3.45 mg/g for loganic acid, 1.11-3.32 mg/g for chlorogenic acid, 0.33-2.82 mg/g for loganin, 1.81-5.47 mg/g for sweroside, 1.22-4.83 mg/g for isochlorogenic acid A, 1.77-26.20 mg/g for sylvestroside I, 2.12-10.24 mg/g for isochlorogenic acid C, 0.33-3.16 mg/g for dipsanosides B, and 0.71-9.52 mg/g for dipsanosides A.

DISCUSSION

PLS-DA was applied to achieve good sample separation. The resulting score plot (Figure 2A) demonstrated that the 32 batches of different medicinal parts from *P. hookeri* could be divided into two groups. In addition, the PLS-DA loading plot (Figure 2B) showed that loganic acid and cantleyoside were the primary chemical biomarkers. To better understand sample contents, graphs were produced from the data in Table 4. Figure 3 shows that compared with the aerial component, the root had significantly higher levels of sylvestroside I, cantleyoside, dipsanosides B, and dipsanosides A, but significantly lower levels of loganic acid, chlorogenic acid, and isochlorogenic acid. There were no significant differences in loganin, sweroside, and isochlorogenic acid C ($p > 0.05$).

Oleanolic acid and ursolic acid are the markers currently used to control *P. hookeri* quality. The 2015 edition of the Chinese Pharmacopoeia dictates that the total contents of oleanolic acid and ursolic acid in should not be less than 0.20 %.

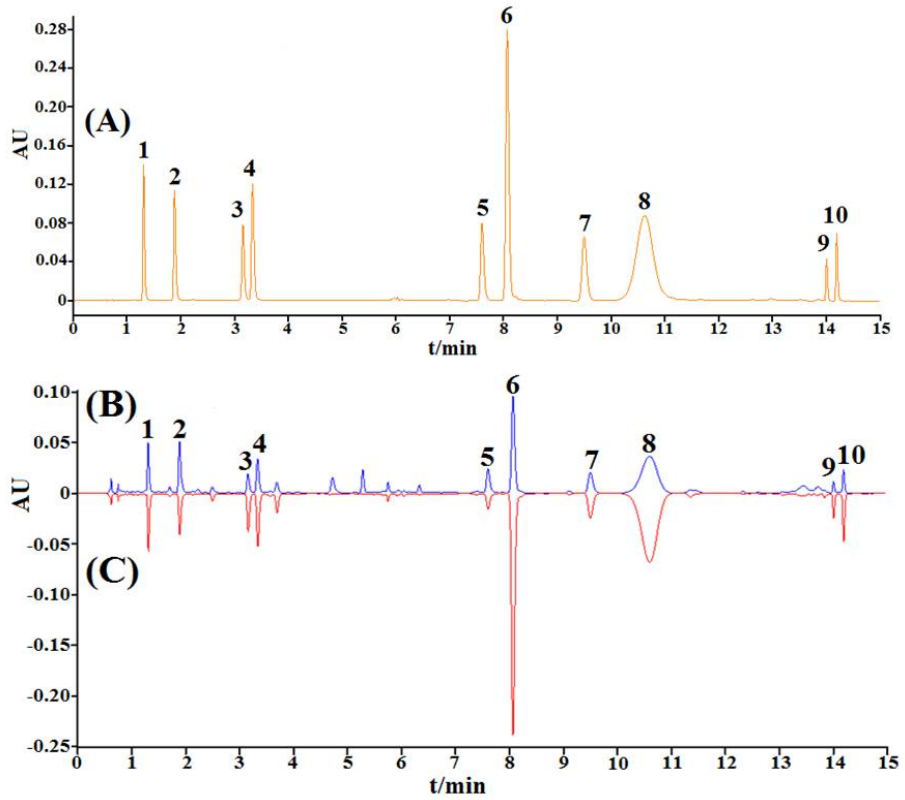

Figure 1: Comparison of UPLC chromatograms for **(A)** standard mixture, **(B)** the aerial part, and **(C)** root of *P. hookeri*. Peaks: 1. loganic acid, 2. chlorogenic acid , 3. loganin, 4. sweroside, 5. isochlorogenic acid A, 6. sylvestroside I, 7. isochlorogenic acid C, 8. cantleyoside, 9. dipsanosides B, 10. dipsanosides A

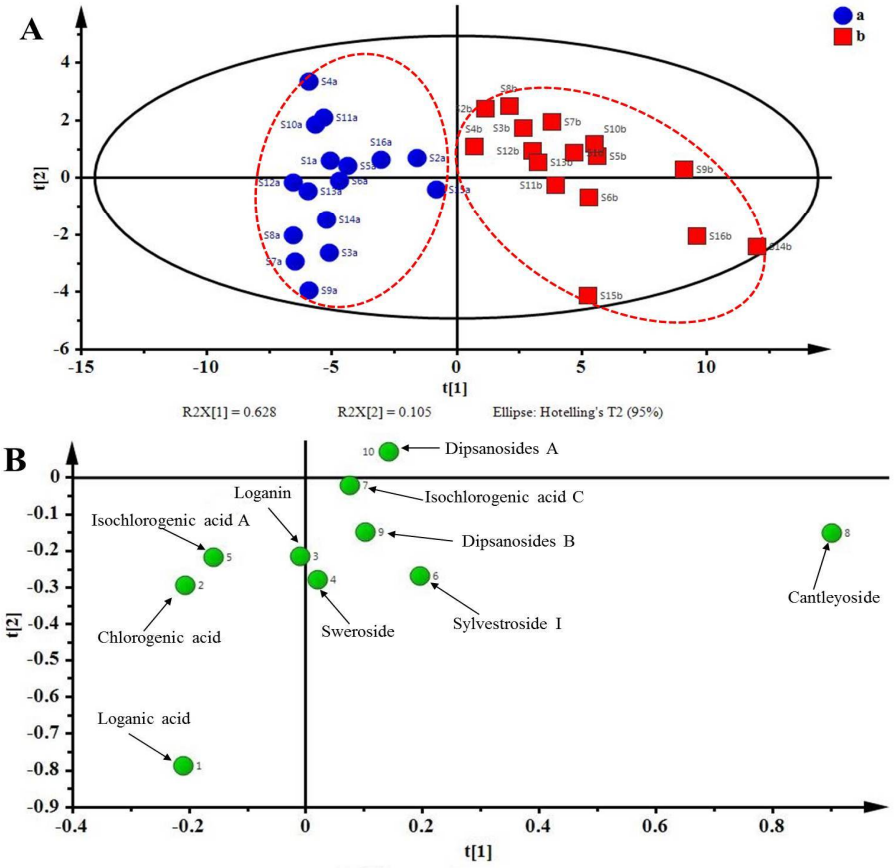

Figure 2: Score (A) and loading (B) plots of PLS-DA of the 32 tested samples derived from UPLC quantitative data. “a”: the aerial part sample, “b”: the root sample

Table 4: Content (mg/g) of 10 compounds in plant parts of *P. hookeri*, determined by UPLC (n = 3)

No.	Voucher number	Sources	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
S1a	2015071500401	Kajia bridge, Luhuo,	5.17	1.73	1.01	2.94	2.61	1.03	2.27	14.24	0.17	0.38
S1b	2015071500402	Sichuan	1.44	1.11	0.82	2.96	1.37	5.56	2.75	62.96	0.33	0.71
S2a	2015071704901	Changgen village, Baiyu,	3.05	1.98	1.68	3.61	1.31	3.87	1.97	29.44	0.61	1.64
S2b	2015071704902	Sichuan	0.39	1.19	0.49	2.27	1.22	5.20	2.12	42.35	0.35	0.72
S3a	2015080500801	Jiangshi country,	9.13	2.36	2.09	5.10	2.15	1.88	1.95	16.33	0.34	0.96
S3b	2015080500802	Kangding, Sichuan	0.80	1.41	0.40	2.57	2.00	4.88	4.16	50.87	0.61	1.25
S4a	2015072100201	Heiga village, Dege,	1.60	0.87	0.51	2.35	1.89	0.54	1.34	6.16	0.23	0.60
S4b	2015072100202	Sichuan	0.65	1.33	1.11	5.47	2.21	5.05	2.28	38.81	1.28	2.30
S5a	2015080501001	Gerima country,	4.73	2.48	1.30	2.94	4.00	0.51	3.95	18.15	0.43	1.23
S5b	2015080501002	Kangding, Sichuan	1.04	2.20	0.54	3.52	3.02	3.16	5.47	68.03	1.02	2.62
S6a	2015071500501	Qianjing village, Luhuo,	6.14	1.39	1.36	3.35	1.76	1.55	2.14	15.60	0.48	1.18
S6b	2015071500502	Sichuan	2.06	1.96	0.90	4.91	2.35	7.99	4.59	64.33	1.09	2.35
S7a	2015080701501	Jiaerduo country, Aba,	4.31	16.70	1.80	3.10	16.34	0.59	7.55	18.53	0.25	0.49
S7b	2015080701502	Sichuan	0.26	1.27	0.42	2.53	2.58	3.30	5.18	58.04	0.37	0.65
S8a	2015080701601	Aqiu country, Aba,	4.78	16.10	0.90	3.71	8.27	0.64	5.27	15.04	0.23	0.54
S8b	2015080701602	Sichuan	0.51	1.31	0.33	1.81	2.00	2.52	5.24	48.66	0.36	0.79
S9a	2015080800201	Xiangmuduo country,	5.33	16.55	2.02	2.92	19.10	0.72	9.79	22.78	0.34	0.84
S9b	2015080800202	Aba, Sichuan	0.53	2.35	0.63	3.11	4.83	2.86	10.24	87.68	0.73	1.81
S10a	2015080801101	Caoyuan country, Aba,	1.60	6.22	0.76	2.04	6.88	0.53	3.93	11.92	0.30	0.86
S10b	2015080801102	Sichuan	0.71	1.21	1.52	3.10	2.63	1.77	5.61	66.91	1.00	3.03
S11a	2015080802801	Exiang country, Aba,	1.69	3.89	0.76	2.40	5.24	1.69	4.24	10.85	0.43	1.24
S11b	2015080802802	Sichuan	2.10	1.93	2.66	3.70	3.30	4.95	9.18	52.68	2.89	9.52
S12a	2015080501801	Yakou, Ruoergai, Sichuan	4.50	5.32	1.13	3.70	6.92	1.54	7.25	6.10	0.67	2.22
S12b	2015080501802		1.46	1.39	1.14	3.78	2.33	4.24	6.26	47.67	2.70	8.87
S13a	2015080900701	Gemo country, Aba,	5.00	8.47	0.81	3.48	2.57	2.46	2.60	11.80	0.24	0.30
S13b	2015080900702	Sichuan	2.10	1.32	1.53	2.84	1.95	4.60	3.37	55.14	0.93	1.47
S14a	2015072101501	Dege country, Aba,	6.00	7.66	1.17	3.52	4.03	3.96	3.49	15.45	0.49	1.05
S14b	2015072101502	Sichuan	2.25	3.32	1.39	3.59	3.39	10.98	6.58	99.29	2.86	5.14
S15a	2015070500401	Xianggelila Tibetan	2.96	3.13	1.46	3.62	2.21	10.86	2.60	31.16	0.74	1.30
S15b	2015070500402	Hospital, Yunnan,	3.45	2.73	2.82	5.44	1.63	26.20	3.30	56.34	1.57	2.57
S16a	2015070601801	Xianggelila, Yunnan	3.32	1.97	0.47	4.95	3.39	1.89	2.88	23.22	0.65	1.15
S16b	2015070601802		2.10	2.67	1.46	5.31	3.10	8.46	5.00	86.33	3.16	5.26

"a": Aerial part sample, "b": root sample. (1): loganic acid, (2): chlorogenic acid, (3): loganin, (4): sweroside, (5): isochlorogenic acid A, (6): sylvestroside I, (7): isochlorogenic acid C, (8): cantleyoside, (9): dipsanosides B. (10):dipsanosides A

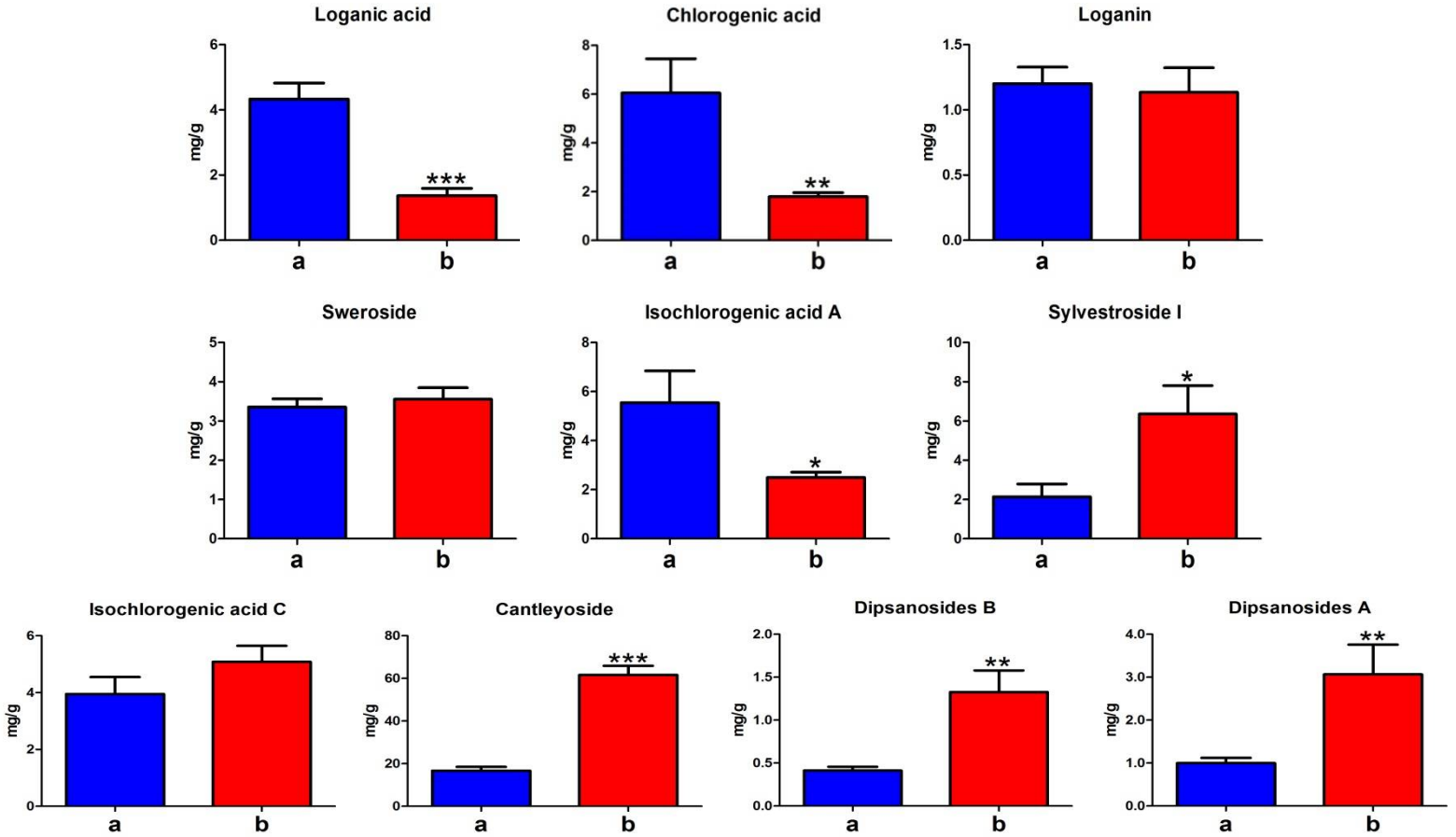

Figure 3: Contents (mg/g) of 10 compounds in different medicinal parts from *P. hookeri* determined by the UPLC method. *, **and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, based on one-way ANOVA tests. “a”: the aerial part sample, n=16; “b”: the root sample, n=16.

P. hookeri samples with lower levels are considered substandard and should not be used as a medicinal material for clinical application. In our previous study [25], we determined the total contents of oleanolic acid and ursolic acid in 32 batches of aerial part samples and found that the contents were all higher than 0.20 %. Interestingly, none of the root samples reached the standard of the 2015 edition of the Chinese Pharmacopoeia.

P. hookeri is a perennial herb that mainly grows at an altitude of 3200-5700 m on hillsides and in grasslands, meadows, and forests. Due to root excavation, it was listed as an endangered Tibetan medicine species in 2000. Understanding how to rationally use *P. hookeri* resources while preventing its depletion is an important issue. Therefore, the results of this study provide quality control references for *P. hookeri*.

CONCLUSION

The developed method is simple, accurate and reproducible, and can be used for the simultaneous determination of the contents of 10 major compounds of *P. hookeri*. The findings demonstrate that there is variation in the chemical composition of the two parts of *P. hookeri*, and that loganic acid and cantleyoside are the main chemical biomarkers.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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