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Simultaneous Determination of Picroside I and II Using High Performance Liquid Chromatography

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Rhizoma Picrorhizae is the dried rhizomes of *Picrorhiza scrophulariaeflora* Pennelland has been used in Chinese medicine for treating fever, liver disorders and jaundice for over a thousand years. The objectives of this research were to determine whether the different regions could result in different quality by determining the content of picroside I and II in different samples. A modified HPLC method using a diode array detector (DAD) has been developed and ten samples of *P. scrophulariaeflora* from eight different regions were evaluated. The results showed that the samples collected from Yunnan in the content of picroside II are higher but lower in the content of picroside I, comparing with the samples collected from Tibet.

Keywords: *Picrorhiza scrophulariaeflora*, Quality evaluation, Different regions, Picroside I, Picroside II, HPLC.

INTRODUCTION

Rhizoma Picrorhizae ("Huhuanglian" in Chinese), the earliest record in "Tang materia medica", is a well known traditional Chinese medicinal (TCM) herb commonly used for curing fever, liver disorders and jaundice for over a thousand years. In the Chinese Pharmacopoeia[1], *P. scrophulariaeflora* are recorded as raw materials of "Huhuanglian". Picrorhiza distributed in the Himalayan region of Sikkim, Nepaland Tibet is a high altitude growing medicinal plants at altitude of 3300-4400 m[2].

Picroside I and II (Fig. 1), the main iridoid glycosides derived from this species were regarded as characteristic constituents and showed significant hepatoprotective activity. Shukla *et al.*[3] reported Picroliv (picroside-I and kutkoside) possessed a more potent choleretic effect in conscious rats and anaesthetised guinea pigs and anticholestatic agent against paracetamol- and ethynylestradiol-induced cholestasis than silymarin. Picroside I and II exhibited a significant reduction in liver lipid content, aminotransferase (AST) and alanine aminotransferase(ALT)[4]. Many researches showed picroside I and II are responsible for hepatoprotective activity[5-7]. Moreover, picroside I and II caused a concentration-dependent enhancement of basic fibroblast growth factor-, staurosporine- and dibutyryl cyclic AMP-induced neurite outgrowth from PC12D cells[8].

Regarding quantitative determination of picroside I and II in genus Picrorhiza, several technologies, including HPLC, UHPLC and HPTLC, have been tried[9-12]. However, to the best of our knowledge no report is available on simultaneous determination of picroside I and II in *P. scrophulariaeflora* from different regions by diode array detector. Therefore, the goal of this study was to establish a reliable HPLC method to determine whether the different regions could result in different quality.

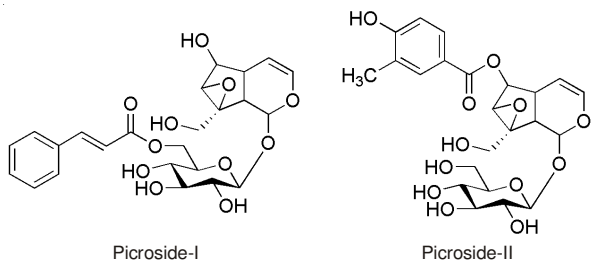

Fig. 1. Structure of picroside-I and picroside-II

EXPERIMENTAL

The materials of *P. scrophulariiflora* identified by Professor Shude Yang, Yunnan university of Yunnan traditional Chinese medicine were collected in October 2012 from eight different regions listed in Table-1. The reference compounds picroside-I (1) and picroside-II (2) were purchased from National Institutes for Food and Drug Control (Beijing, China). Methanol (HPLC-grade) was purchased from Merck (Darmstadt, Germany) and Water was purified using a Millipore simplicity system.

TABLE-1
SUMMARY FOR THE DIFFERENT REGIONS OF *P. scrophulariaeflora*

No.	Growing regions	Parts	Altitude (m)
1	Baimaxueshan, Yunnan (BM)	Rhizomes	4040
2	Cizhong, Yunnan (CZ)	Rhizomes	3620
3	Sinong, Yunnan (SN)	Rhizomes	3650
4	Yongzhi, Yunnan (YZ)	Rhizomes	3900
5	Dingri, Tibet (DR)	Rhizomes	4248
6	Yadong, Tibet (YD)	Rhizomes	4360
7	Nielamu, Tibet (NLM)	Rhizomes	4050-4300
8	India	Rhizomes	3810
9	Sinong, Yunnan (SNL)	Leaves	3650
10	Cizhong, Yunnan (CZL)	Leaves	3620

Sample preparation: The air-dried rhizomes or leaves (100 mg) of *P. scrophulariaeflora* were extracted with ultrasonication in 50 mL methanol for 0.5 h. The extracts were filtered through 0.45 µm Millipore membrane, 1 mL of the filtrate was dilute with methanol to 5 mL and 10 µL of each sample was used for HPLC analysis.

Chromatographic conditions: HPLC, Dionex. (UltiMate 3000, Sunnyvale, USA) was equipped with, an auto-sample, a quaternary pump system and a DAD detector. All samples and standards were filtered through 0.45 µm (Millipore) filters. Separation was achieved on Kromat Universil C18 (4.6 × 250 mm, 5 µm). The mobile phase was consisted of methanol: 0.2 % phosphoric acid water (41:59, v/v) isocratic elution with the flow rate mL min^{-1}. The column temperature was kept 20 °C.

Method validation: The methanol stock solution of standards was prepare and diluted with methanol to appropriate concentrations for establishment of calibration curve. At least twelve concentrations of picroside I and II were injected in triplicate, respectively for the construction of calibration curves. The limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined at S/N (of signal-to-noise ratio) of 3 and 10, respectively.

Intra-day variation was chosen to determine the precision of the method. The known concentrations of two standard solutions were tested. For intra-day variability test, the standard solutions were analyzed seven times within one day. Recovery test was used to evaluate the accuracy of the method. For the percent recovery experiments, three different amounts of picroside I and II were spiked to the three samples from Sinong, Yunnan. The spiked samples were extracted and analyzed by the proposed method.

RESULTS AND DISCUSSION

The proposed method for quantitative analysis of picroside I and II was validated in terms of linearity, accuracy, repeatability and stability when compared with standard picroside I and II. Linearity was examined with a standard solution prepared in the range of 0.0446-1.1596 and 0.0818-1.0634 µg of picroside I and II, respectively. The linear relationship between the amounts (µg, x-axis) and peak area ratio (*y*-axis) was expressed by the following equation: Y = 24.113X-0.7528 (picroside I) and Y = 7.8585X - 0.0.2337 (picroside II). The correlation coefficient was 0.9997 and the calibration curve was a straight line. The LODs (S/N = 3) and LOQs (S/N = 10) for picroside I and II were 0.0182, 0.06059 µg and 0.0195, 0.06503, respectively (Table-2).

The accuracy was confirmed by performing a recovery experiment, where one sample was spiked with known amounts of picroside I and II (Table-3). The mean recovery rates ware 102.48 % (picroside I) and 100.77 % (picroside II) and the relative standard deviation (RSD) were 1.53 % (picroside I) and 3.48 % (picroside II). The precision results (Table-4) showed the low values (less than 2 %) of intra-day % RSD of peak areas. Therefore, the developed method is precise, accurate and enough sensitive for simultaneous quantitative evaluation of picroside I and II in different regions.

Quantitation of picrosides: The content of picroside I and II in the eight rhizome samples and two leaf samples of *P. scrophulariaeflora* collected from different geographic areas were determined using the above developed HPLC method (Table-5). Because *P. scrophulariaeflora* are recorded in the Chinese Pharmacopoeia and can be used in clinical practice, the picroside I and II content this plant was determined. Picroside-I was observed in higher amount (10.46-16.47 %) as compared to picroside-II (1.12-2.24 %) collected from Yunnan. The samples collected from Sinong, Yunnan (3650 m) ware found to contained larger amount of picroside-I (16.47 %) and total picrosides content (18.91 %). The order of total picrosides content was Sinong, Yunnan > Baimaxueshan, Yunnan > Yadong, Tibet > Cizhong, Yunnan > India > Nielamu, Tibet. Additionally, leaf samples collected from Sinong, Yunnan and Cizhong, Yunnan, Yunnan ware found to contained large amount of total picrosides.

Conclusion

A convenient and reliable HPLC method using a DAD detector has been developed for quantitative analysis of picroside

TABLE-2
LINEAR REGRESSION DATA OF INVESTIGATED COMPOUNDS FROM *P. scrophulariaeflora*

Compounds	Regression equation	Linearity range (µg)	r^2	LOD (µg)	LOQ (µg)
Picroside I	y = 24.113x - 0.7528	0.0046-1.1596	0.9997	0.0182	0.06059
Picroside II	y = 7.8585x - 0.0.2337	0.0818-1.0634	0.9997	0.0195	0.06503

TABLE-3
PRECISION PEAK AREAS FROM *P. scrophulariaeflora*

Analytes	Intra-day precision							RSD (%)
	0	1	2	4	8	12	24	
Picroside I	9.775	9.779	9.725	9.838	9.769	9.736	9.812	0.40
Picroside II	2.945	2.986	2.923	3.051	3.087	3.089	3.067	2.28

TABLE-4
RECOVERY STUDY OF PICROSIDE I AND II BY THE HPLC METHOD

Analytes	Amount of sample (mg)	Spiked amount (mg)	Amount found in mixture (mg)	Recovery (%)	RSD (%)
Picroside I	0.1007	3.05	6.054	102.48	1.53
	0.1010	2.55	5.085		
	0.1024	2.64	5.155		
Picroside II	0.0259	3.35	7.65	100.77	3.48
	0.0265	3.05	7.29		
	0.0251	3.13	7.44		

TABLE-5
CONTENTS (%) OF PICROSIDE-I, PICROSIDE-II FROM, DIFFERENT REGIONS

No.	Growing regions	Picroside-I (%)	Picroside-II (%)	Picrosid I and II (%)
1	BM	1.12	15.61	16.73
2	CZ	1.30	12.18	13.48
3	SN	-	6.93	-
4	YZ	2.44	16.47	18.91
5	DR	5.15	8.89	14.04
6	YD	-	15.61	-
7	NLM	3.64	12.18	9.01
8	India	2.57	6.93	10.62
9	SNL	11.08	16.47	18.44
10	CZL	9.43	8.89	12.04

I and II content in the plant of *P. scrophulariaeflora*. This analytical method was validated by its good linearity, accuracy and precision. Utilizing this technology, we have successfully determined the content of picroside I and II in ten samples of the plants. There was a very large variation in the content of picroside I and II among the plants from different regions, which implies that the region has great impact on the quality of *P. scrophulariaeflora* in terms of the content of picroside I and II. Moreover, the samples collected from Yunnan in the content of picroside I are higher but lower in the content of picroside II, comparing with the samples collected from Tibet. Even though the leaves of *P. scrophulariaeflora* showed high content of total picrosides, the more research should be performed to prove whether it would be regarded as the substitution of rhizomes.

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