Quality Assessment of Fructus Psoraleae

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Received November 14, 2005; accepted March 11, 2006

Two newly-reported benzofuran glycosides, named psoralenoside and isopsoralenoside, along with two major coumarins, psoralen and isopsoralen, were simultaneously determined in twenty-three samples of Fructus Psoraleae collected from different growth areas in China. The quantitative method was validated, and the mean recovery rates from fortified samples ($n=5$) of psoralenoside, isopsoralenoside, psoralen and isopsoralen, were 96.5%, 97.1%, 100.7%, and 99.3% with variation coefficient of 3.1%, 3.6%, 2.3%, and 2.2%, respectively. An interesting biotransformation relationship between the glycosides and the coumarins was revealed on the basis of the quality analysis results. It was also suggested that psoralenoside and isopsoralenoside should be used as key quality markers for Fructus Psoraleae, together with the commonly used psoralen and isopsoralen.

Key words Fructus Psoraleae; *Psoralea corylifolia*; psoralenoside; isopsoralenoside; quality assessment

Fructus Psoraleae, the dried fruits of *Psoralea corylifolia* L., is one of the most popular traditional Chinese medicine and is officially listed in the Chinese Pharmacopoeia. This crude drug has been used for the treatment of enuresis, pollakiuria, weak kidney, and pain and cold in the waist and knees. It always attracts research interest from various related fields. Last year, over ten articles have been published with focus on its quality analyses,[1—3] phytochemistry,[4—8] and bioactivities.[9—16] Our previous phytochemical investigation on this medicinal herb revealed two new benzofuran glycosides, psoralenoside (**1**) and isopsoralenoside (**2**), using high performance liquid chromatography mass spectroscopy (HPLC-MS) technique.[17] They were found to have interesting structures which were related to the major constituents of Fructus Psoraleae, psoralen (**3**) and isopsoralen (**4**). From the chemistry point of view, psoralen and isopsoralen could be the products of the hydrolyzation of these two new benzofuran glycosides. However, all the previously reported quality analyses of this important herbal medicine were focused on coumarins (**3**) and (**4**). Therefore, we carried out a new quality research using both glycosides (**1**) and (**2**) and coumarins (**3**) and (**4**) as the quality markers, which led to the discovery of some attractive relationships between these chemical markers.

Experimental

Reagents and Materials Twenty-three batches of the raw material of Fructus Psoraleae were collected from different regions of China and they were authenticated as genuine *P. corylifolia* according to their morphological characteristics. The voucher specimens were deposited in the Chinese Medicine Laboratory of Hong Kong Jockey Club Institute of Chinese Medicine. The reference chemical standards of psoralen (batch No. 110739-200309) and isopsoralen (batch No. 110738-200410) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Psoralenoside and isopsoralenoside were isolated and purified as described in our previous report.[17] The purities of the chemical references were determined to be beyond 98% by HPLC analysis. HPLC grade methanol and acetic acid were purchased from International Laboratory (Nevada, U.S.A.). HPLC grade water was prepared by Millipore Milli-Q SP water purification system.

Apparatus and Column The Agilent 1100 series HPLC system equipped with a Zorbax® XDB-C_8 analytical column (4.6×150 mm, 5 μm, Agilent Technologies, U.S.A.), a C_{18} guard column (4.6×12.5 mm, 5 μm, Agilent Technologies, U.S.A.) and a photodiode array detector (DAD) was set up for the analysis. The analysis was performed at 20 °C during the whole process. The mobile phase was a mixture of methanol and water (containing 0.1% acetic acid) at a flow rate of 1.0 ml/min. Linear gradient elution from 25 to 60% methanol (v/v) in 30 min was applied. The detection wavelength was set at 246 nm.

Standard Solutions The stock solutions were prepared in methanol at the concentrations of 0.636 mg/ml (psoralen), 0.588 mg/ml (isopsoralen), 0.611 mg/ml (psoralenoside) and 0.587 mg/ml (isopsoralenoside), respectively. The stock solutions were diluted and mixed in 5 ml volumetric flasks to yield a series of standard solutions with different concentrations for the validation of linearity.

Sample Preparation Samples were pulverized and the powder was screened through 355 μm sieves. The fine powder (1 g) was accurately weighed, and mixed with 50 ml methanol in a flask (100 ml). The flask was weighed again. Then, the powder was refluxed in methanol for 4 h. After cooling, methanol was added to make up to the initial weight. The supernatant fluid was filtered through a syringe filter (0.45 μm). Five microliters of this solution was injected into the HPLC system for analysis. All the standard solutions and sample solutions can be stored at 4 °C in one week without quality loss.

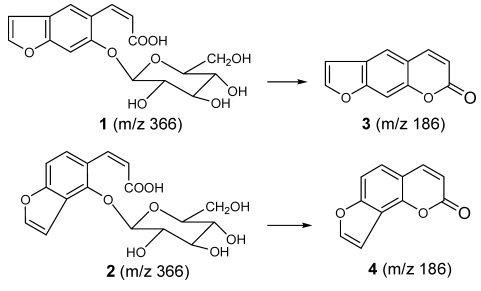

Fig. 1. Chemical Transformation of **1** and **2** to **3** and **4**

Results and Discussion

A total of 23 samples (samples 1—23) of Fructus Psoraleae were collected from different regions of China (Table 1). The HPLC chromatograms were generated at wavelengths of 246 nm (Fig. 2). The peaks of psoralenoside (**1**), isopsoralenoside (**2**), psoralen (**3**), and isopsoralen (**4**) in these samples were identified by comparison with the reference chemical standards.

Validation of the Quantitative Analysis The proposed method for quantitative analysis of four constituents in Fructus psoraleae was validated in terms of linearity, recovery

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Table 1. Contents (mg/100 mg, Mean±S.D., n=3) of **1**—**4** in 23 Batches of Fructus Psoraleae

Sample No.	Purchased markets (growth area)	1	2	3	4
1	Chongqing (Chongqing)	3.02±0.05	2.68±0.06	0.05±0.01	0.05±0.01
2	Chongqing (Chongqing)	2.55±0.02	2.39±0.04	0.15±0.02	0.16±0.01
3	Nanjing, Jiangsu (Yunnan)	2.33±0.03	1.99±0.02	0.18±0.02	0.16±0.01
4	Chongqing (Chongqing)	2.24±0.02	1.96±0.01	0.14±0.02	0.10±0.02
5	Shenzhen, Guangdong (Sichuan)	2.07±0.04	1.76±0.03	0.25±0.04	0.22±0.03
6	Hong Kong (unknown)	1.95±0.02	1.66±0.07	0.30±0.03	0.27±0.02
7	Nanjing, Jiangsu (Yunnan)	1.89±0.04	1.56±0.02	0.29±0.01	0.27±0.01
8	Nanjing, Jiangsu (Anhui)	1.87±0.01	1.59±0.01	0.26±0.03	0.23±0.02
9	Hong Kong (unknown)	1.84±0.05	1.51±0.02	0.25±0.05	0.22±0.02
10	Hong Kong (Guizhou)	1.75±0.03	1.46±0.03	0.35±0.03	0.34±0.03
11	Shuozhou, Shanxi (Liaoning)	1.75±0.03	1.49±0.03	0.41±0.02	0.36±0.04
12	Nanjing, Jiangsu (Guangxi)	1.73±0.03	1.45±0.03	0.37±0.05	0.32±0.05
13	Xinxiang, Henan (Henan)	1.69±0.02	1.44±0.01	0.42±0.03	0.38±0.02
14	Hong Kong (Guangxi)	1.55±0.04	1.32±0.04	0.49±0.02	0.45±0.03
15	Shuozhou, Shanxi (Liaoning)	1.55±0.01	1.39±0.04	0.46±0.04	0.43±0.04
16	Shenzhen, Guangdong (unknown)	1.32±0.06	1.10±0.05	0.44±0.01	0.40±0.02
17	Wuhan, Hubei (unknown)	1.29±0.03	1.03±0.04	0.48±0.02	0.40±0.03
18	Shenzhen, Guangdong (unknown)	1.02±0.01	0.85±0.02	0.55±0.03	0.55±0.03
19	Hong Kong (unknown)	0.95±0.01	0.82±0.02	0.67±0.03	0.61±0.05
20	Nanjing, Jiangsu (Liaoning)	0.85±0.01	0.69±0.03	0.68±0.04	0.60±0.04
21	Shenzhen, Guangdong (unknown)	0.76±0.04	0.66±0.01	0.69±0.04	0.61±0.05
22	Nanjing, Jiangsu (Yunnan)	0.73±0.02	0.60±0.04	0.68±0.03	0.64±0.05
23	Shenzhen, Guangdong (unknown)	0.30±0.03	0.26±0.02	0.95±0.05	0.87±0.04

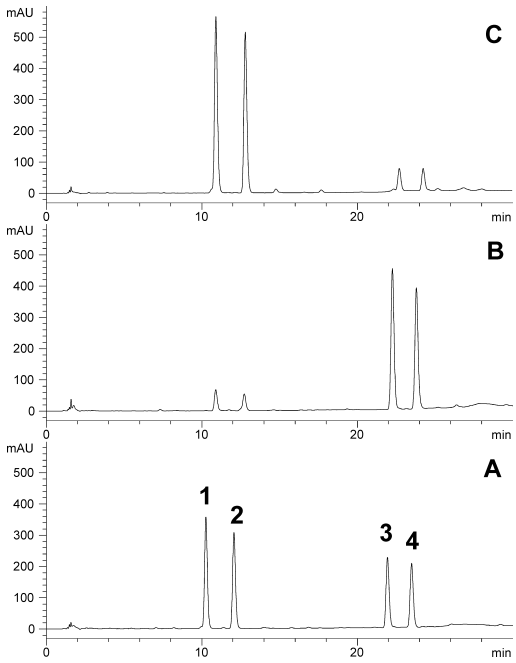

Fig. 2. Typical HPLC Chromatogram of **1**—**4** in Fructus Psoraleae
(A) reference standards, (B) sample No. 23, (C) sample No. 1.

and repeatability. Linearity was examined with standard solutions. The linear relationships between the injection quantities (μg, x-axis) and peak area ratio (y-axis) were expressed by the equations listed in Table 2. The correlation coefficients ranged from 0.9997 to 0.9999, and the calibration curves were straight lines. Quintuplicate samples were spiked with known amounts of these four compounds and then extracted in order to study the recoveries. The average recoveries ranged from 96.47 to 100.73%, and the ranges of relative standard deviations (RSD) were from 2.19 to 3.57%. Five individual samples from the same batch were extracted and processed in accordance with sample preparation procedures for quantitative analysis. The RSD of the determination results of these four constituents ranged from 1.98 to 3.03%. It indicated that the repeatability is suitable for quantitative analysis. The validation results (calibration equation, recovery and repeatability) are listed in Table 2.

Determination of Compounds 1—4 The contents of psoralenoside (**1**), isopsoralenoside (**2**), psoralen (**3**), and isopsoralen (**4**) in the 23 samples listed in Table 1 were determined by HPLC (Fig. 2). The results showed that the tested samples contained very different contents of psoralen (**3**), and isopsoralen (**4**), ranging from 0.05 to 0.95%, and from 0.05 to 0.87%, respectively. As shown in Fig. 2, sample No. 1 contained the lowest amount of coumarins **3** and **4**, while sample No. 23 had the highest (almost twenty times higher). The coumarin contents were rising from sample No. 1 to No. 23 (Fig. 3). According to these results, sample No. 23 would usually be regarded as the best material, and sample No. 1 would be the worst. However, the determination of the benzofuran glycoside led to a more interesting discovery. From sample No. 1 to No. 23, the contents of psoralenoside (**1**), and isopsoralenoside (**2**) dropped from the top at 3.02 and 2.68% to the bottom at 0.30 and 0.26%, respectively (Fig. 3). A completely different conclusion could be made on the basis of this interesting discovery: sample No. 1 being the best material, and sample No. 23 the worst material. These were two different quality assessment methods by different chemical markers. It would be more considerable to combine them.

A new parameter, namely the converted content (CC), was therefore introduced from the biosynthetic relationship between coumarins **3** and **4** with the benzofuran glycosides **1** and **2** (Fig. 1). This new concept indicated the potential content of the coumarins that were derived from the related benzofuran glycosides in a ratio of their molecular weight 186/366, *e.g.*, $CC_{1\rightarrow3}=C_1\times186/366$. As a consequence, the total content of **3** which included the determined content (C_3) and the potential content was calculated as shown in Table 3

Table 2. Validation Data of the Quantitative Analysis of Fructus Psoraleae by HPLC

Compound	Calibration equation	Correlation coefficient	Average recovery (%)	Recovery RSD (%)	Repeatability RSD (%)
1	$y=2949.3x+6.0649$	0.9998	96.5	3.1	2.5
2	$y=2924.7x+13.999$	0.9999	97.1	3.6	3.0
3	$y=6789.9x-14.593$	0.9998	100.7	2.3	2.0
4	$y=6714.4x-12.456$	0.9997	99.3	2.2	2.0

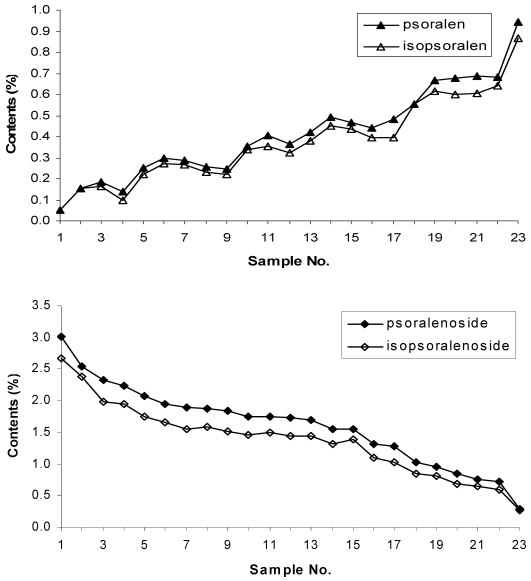

Fig. 3. Contents of Psoralen, Isopsoralen, Psoralenoside, and Isopsoralenoside Determined in 23 Samples

Table 3. Converted Contents (from **1** and **2** to **3** and **4**) and Total Contents of Psoralen (**3**) and Isopsoralen (**4**) in 23 Batches of Fructus Psoraleae

Sample	Converted content		Total content	
	$CC_{1\to3}$	$CC_{2\to4}$	$C_{total\text{-}3}$	$C_{total\text{-}4}$
1	1.53	1.36	1.58	1.41
2	1.30	1.21	1.45	1.37
3	1.19	1.01	1.37	1.17
4	1.14	1.00	1.28	1.09
5	1.05	0.89	1.30	1.11
6	0.99	0.84	1.29	1.12
7	0.96	0.79	1.25	1.06
8	0.95	0.81	1.21	1.04
9	0.94	0.77	1.18	0.99
10	0.89	0.74	1.24	1.08
11	0.89	0.76	1.30	1.11
12	0.88	0.74	1.25	1.06
13	0.86	0.73	1.28	1.11
14	0.79	0.67	1.28	1.12
15	0.79	0.70	1.25	1.14
16	0.67	0.56	1.11	0.95
17	0.65	0.52	1.14	0.92
18	0.52	0.43	1.07	0.99
19	0.48	0.42	1.15	1.03
20	0.43	0.35	1.11	0.95
21	0.38	0.33	1.07	0.94
22	0.37	0.30	1.05	0.95
23	0.15	0.13	1.10	1.00

based on the formulae $C_{total\text{-}3}=C_3+CC_{1\to3}$. Similarly, the total content of **3** was also calculated. It was revealed by the calculated results that all twenty-three samples (Fig. 4) contained relatively reliable contents of coumarins **3** and **4** (about 1.20%, 1.05%, respectively). In other words, the Fructus Psoraleae with low contents of **1** and **2**, and high contents of **3** and **4**, *e.g.* sample No. 1, and those with high contents of **1** and **2**, and low content **3** and **4**, *e.g.* sample No. 23, might have the same biological activity, and their quality would not be very changeable, if a processing method was applied to convert all the glycosides **1** and **2** into the coumarins **3** and **4**. This suggested that the yielding time and storage conditions rather than the culturing area had considerable effects on the quality of Fructus Psoraleae. In conclusion, it is strongly recommended that along with the major coumarins, psoralen and isopsoralen, these two newly-reported benzofuran glycosides, psoralenoside and isopsoralenoside, should be set as chemical markers in the quality control and quality analysis of Fructus Psoraleae.

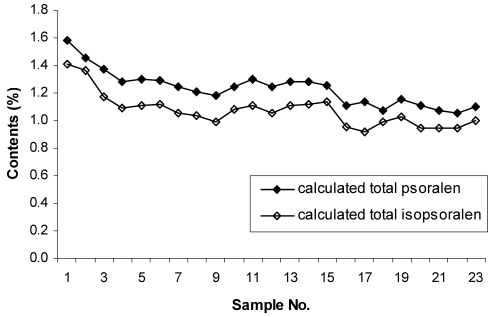

Fig. 4. Total Contents of Psoralen and Isopsoralen Calculated in 23 Samples

Acknowledgments This research is funded by the Hong Kong Jockey Club Charities Trust.

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