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Analysis of Bakuchiol, Psoralen and Angelicin in Crude Drugs and Commercial Concentrated Products of Fructus Psoraleae

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ABSTRACT

The goal of this study was to develop a HPLC method for simultaneously determining bakuchiol, psoralen and angelicin in Fructus Psoraleae (Buguzhi, the fruits of *Psoralea corylifolia*) and its commercial concentrated products. Extracted samples were analyzed by using a reverse-phase column (Cosmosil 5C18-AR-II) and eluting with a gradient mobile phase consisting of 20% acetonitrile to acetonitrile at a flow rate of 1.0 mL/min with detection at a wavelength of 254 nm. The relative standard deviations of the marker substances on the basis of peak-area ratios in intraday and interday analyses were 0.3~1.5% and 0.2~1.0%, respectively. Bakuchiol, psoralen and angelicin contents were 36.2~71.0, 2.5~13.0 and 2.2~9.2 mg/g for ten raw material samples of Fructus Psoraleae, and 0.6~21.1, 0.6~5.2 and 0.6~5.3 mg/g for eight commercial concentrated products of Fructus Psoraleae, respectively.

Key words: *Psoralea corylifolia*, HPLC, bakuchiol, psoralen, angelicin

INTRODUCTION

Psoralea corylifolia L. (Fructus Psoraleae, Chinese name Buguzhi) fruits are used in Chinese medicine for the treatment of premature ejaculation, spermatorrhea, enuresis, backache, knee pain, pollakiuria, vitiligo, callus, psoriasis and alopecia[(1)]. Some coumarins, flavonoids and terpenoids have been isolated from this plant[(1-4)]. Bakuchiol (Figure 1), an important monoterpene phenol, is abundant in the seeds and has been reported to possess DNA polymerase inhibitory activity and show antitumor, antibacterial, cytotoxic, anti-helminthic, and anti-oxidant activities[(5-7)]. Psoralen and angelicin also show antibacterial and phototherapeutic activities (Figure 1)[(8,9)]. Although analytical methods for Fructus Psoraleae have been established[(10-14)], these methods cannot simultaneously quantify these three active compounds. In this study we isolated three marker substances, i.e. bakuchiol, psoralen, and angelicin, from Fructus Psoraleae and simultaneously measured the contents of these three active compounds in raw material of Fructus Psoraleae and the commercial concentrated products of Fructus Psoraleae in a single run using a reverse phase HPLC method with gradient elution.

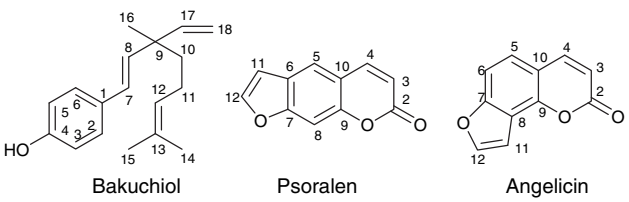

Figure 1. Structures of bakuchiol, psoralen and angelicin.

MATERIALS AND METHODS

I. *Reagents and Materials*

Ten batches of the raw material of Fructus Psoraleae were collected from different herbal import companies in Taiwan. Eight commercial concentrated products of Fructus Psoraleae were purchased from eight different factories in Taiwan. Bakuchiol, psoralen and angelicin were isolated from Fructus Psoraleae in our laboratory, and 2-(4-hydroxyphenyl)ethyl alcohol was purchased

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from Sigma (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were obtained from Mallinckrodt (Phillipsburg, NJ, USA). Ultra-pure distilled water with a resistivity greater than 18 MΩ was produced by a Milli-Q system (Millipore, Bedford, USA). Samples for HPLC were filtered through a 0.45 μm membrane filter (Millipore, Bedford, USA). Melting points were determined on a Yanaco MP-I3 micro melting point apparatus. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Unity INOVA 500 or a Bruker AMX-400 NMR spectrometer. EIMS spectra were obtained using a Finnigan MAT GCQ spectrometer.

II. *Isolation of Marker Substances*

Pulverized Fructus Psoraleae (1.0 kg) was heated for 2 hr in 5.0 L of Me_2CO at 50°C and filtered. The filtrate was then condensed under vacuum to give a dry residue. The residue was chromatographed on a silica gel (70-230 mesh) column using *n*-hexane-EtOAc mixture as the eluting solvent. The fraction, eluate of *n*-hexane-EtOAc (15:1), was chromatographed on a Sephadex LH-20 column (eluting with MeOH) to give bakuchiol (8.5 g). The eluate of *n*-hexane-EtOAc (1:1) was repeatedly chromatographed on silica gel [230-400 mesh, eluting with *n*-hexane-EtOAc (1:1)] and Sephadex LH-20 (eluting with MeOH) to give psoralen (3.2 g) and angelicin (3.4 g).

Bakuchiol (1) [17,18] brown liquid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26 (3H, s, H-16), 1.56 (2H, m, H-10), 1.65 (3H, s, H-15), 1.74 (3H, s, H-14), 2.02 (2H, m, H-11), 5.08 (2H, m, H-18), 5.17 (1H, br t, H-12), 5.94 (1H, dd, J = 17.3, 10.8 Hz, H-17), 6.11 (1H, d, J = 16.0 Hz, H-8), 6.25 (1H, d, J = 16.0 Hz, H-7), 6.82 (2H, d, J = 8.5 Hz, H-3, 5), 7.28 (2H, d, J = 8.5 Hz, H-2, 6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.6 (C-15), 23.2 (C-16), 23.3 (C-11), 25.6 (C-14), 41.2 (C-10), 42.4 (C-9), 111.8 (C-18), 115.4 (C-3 and C-5), 124.8 (C-12), 126.4 (C-7), 127.3 (C-2 and C-6), 130.8 (C-1), 131.2 (C-13), 135.8 (C-8), 145.9 (C-17), 154.5 (C-4); EIMS *m/z* (rel int) 256 (53, $[M]^+$), 241 (15), 213 (50), 186 (23), 173 (100), 158 (16), 145 (71), 107 (24).

Psoralen (2) [10,16,17] colorless needles from MeOH; mp 162-163°C; 1H NMR (400 MHz, $CDCl_3$) δ, 6.37 (1H, d, J = 9.6 Hz, H-3), 6.81 (1H, d, J = 2.4 Hz, H-11), 7.46 (1H, s, H-8), 7.66 (1H, s, H-5), 7.67 (1H, d, J = 2.4 Hz, H-12), 7.79 (1H, d, J = 9.6 Hz, H-4); EIMS *m/z* (rel int) 186 (100, $[M]^+$), 158 (53), 130 (12), 102 (14).

Angelicin (3) [10,15,17] colorless needles from MeOH; mp 137-138°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (1H, d, J = 9.6 Hz, H-3), 7.12 (1H, d, J = 2.0 Hz, H-11), 7.36 (1H, d, J = 8.5 Hz, H-5), 7.42 (1H, d, J = 8.5 Hz, H-6), 7.68 (1H, d, J = 2.0 Hz, H-12), 7.79 (1H, d, J = 9.6 Hz, H-4); EIMS *m/z* (rel int) 186 (100, $[M]^+$), 158 (62), 130 (14), 102 (15).

III. *Preparation of Samples*

One gram of each pulverized Fructus Psoraleae sample was heated in methanol (50 mL) for 1 hr and filtered. The filtrate volume was adjusted to 100 mL with methanol. One milliliter aliquot of the methanolic solution and 1 mL of internal standard solution [IS, 1 g of 2-(4-hydroxyphenyl)ethyl alcohol in 100 mL MeOH] were mixed and adjusted to 10 mL with methanol. Five grams of commercial concentrated products were treated as pulverized Fructus Psoraleae and diluted with an appropriate volume of methanol. After filtration (0.45 μm), 20 μL of the filtrate was injected into the HPLC system. Water and 50% methanol were used as extraction solvents for comparison.

IV. *Calibration Curves*

Stock solutions of bakuchiol, psoralen and angelicin were prepared at the concentrations of 175.0, 116.7 and 100.0 μg/mL, respectively. The stock solutions were diluted to yield a series of standard solutions with different concentrations to construct a calibration curve. To each of the standard solutions, 1 mL of internal standard solution was added. The calibration curves were prepared by plotting the linear regression of the peak-area ratios (y) vs. concentrations (x, μg/mL) of each constituent.

V. *Condition of HPLC Analysis*

HPLC was conducted on a HP model 1100 system equipped with a HP G1311A QuatPump, an HP G1322A degasser and a HP G1315B photodiode array detector set at 254 nm. The separations involved the use of a reversed phase column (Cosmosil 5C18-AR-II, 5 μm, 4.6 mm I.D. × 250 mm) and a linear gradient from 20% aqueous acetonitrile to 100% acetonitrile for 30 min at a flow rate of 1.0 mL/min.

VI. *Suitability Evaluation*

(I) *Reproducibility Test*

Standard stock solutions with various concentrations of bakuchiol (17.5, 35.0 and 175.0 μg/mL), psoralen (1.7, 14.5 and 116.7 μg/mL) and angelicin (1.5, 12.5 and 100.0 μg/mL) were used for the reproducibility tests. Intraday and interday analyses on precision were performed using the optimum HPLC conditions.

(II) *Recovery of Bakuchiol, Psoralen, and Angelicin*

For the accuracy study, three different levels of bakuchiol (17.5, 35.0 and 70.0 mg), psoralen (5.0, 10.0 and 20.0 mg) and angelicin (5.0, 10.0 and 20.0 mg) were added to a selected sample of crude drug (1.00 g each). Pulverized Fructus Psoraleae (each 1.0 g) was mixed with an appropriate volume of standard stock solution, and then heated with reflux in methanol (50 mL) for 1 hr and filtered. The filtrate was adjusted to 100 mL with methanol. One milliliter of the solution and 1 mL of internal

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Table 1. Regression equations, correlation coefficients (r) and limits of detection (LOD) of bakuchiol, psoralen, and angelicin

Compound	Concentration (μg/mL)	Regression equation	r	LOD (ng/mL)
Bakuchiol	17.5~175.0	y = 0.0248x - 0.1284	0.9999	175.0
Psoralen	1.7~116.7	y = 0.0386x - 0.0362	0.9998	37.5
Angelicin	1.5~100.0	y = 0.0423x - 0.0363	0.9995	43.7

standard solution were then mixed and diluted to 10 mL with methanol. Each sample was filtered through a 0.45 μm membrane filter and analyzed for the contents of bakuchiol, psoralen, and angelicin as described above. The recovery (%) was caculated as $[(C_{spike} - C_{drug})/C_{std}] \times 100\%$, where C_{spike} is the total content of bakuchiol, psoralen or angelicin in the drug (Fructus Psoraleae) spiked with the standard (C_{std}) and C_{drug} is the content of bakuchiol, psoralen, and angelicin in the drug.

(III) *Determination of the Limit of Detection (LOD)*

The standard stock solutions were diluted with methanol to provide a series of solutions with the appropriate concentrations. The limits of detection of this method were determined by measuring the signal-to-noise ratio for each compound by injecting a series of solutions until the S/N ratio reached 3.

RESULTS AND DISCUSSION

I. *Isolation of Marker Substances*

Three marker substances, bakuchiol, psoralen, and angelicin, were isolated from Fructus Psoraleae and identified by comparison of their MS and NMR spectral data with literature values[10,15-18]. The purity of each marker was greater than 98% as indicated by HPLC with photodiode array (PDA) detector and NMR analysis.

II. *Separation Results by HPLC*

Through a series of experiments, we found 2-(4-hydroxyphenyl)ethyl alcohol to be a suitable internal standard for the assay of Fructus Psoraleae and the water-acetonitrile elution solvent to be a good mobile phase. A photodiode array detector was used to determine the optimized conditions for the marker substances in the chromatogram. In a full-scan experiment, the detector wavelength at 254 nm showed better separation than other wavelengths. HPLC chromatogram of a methanol extract of Fructus Psoraleae with the internal standard is shown in Figure 2. No interfering signals to the marker substances were observed. The retention times for the internal standard, angelicin, psoralen, and bakuchiol were 4.86, 13.90, 14.36, and 28.17 min, respectively.

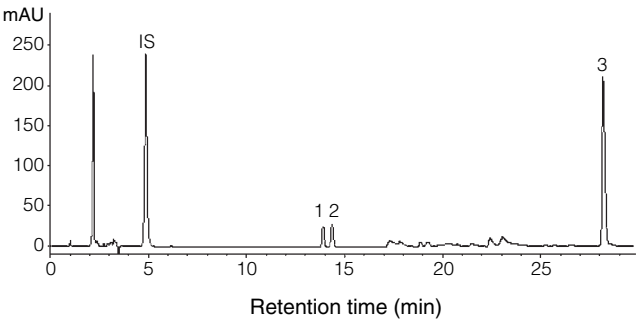

Figure 2. HPLC chromatogram of Fructus Psoraleae. 1: angelicin, 2: psoralen, 3: bakuchiol, IS: 2-(4-hydroxyphenyl)ethyl alcohol.

III. *Selection of Extraction Solvent*

In order to increse the extraction efficiency of the marker substances from Fructus Psoraleae, three extraction solvents, water, 50% methanol, and methanol, were compared. Methanol was found to be the best extraction solvent for these three marker substances. When water was used as the extraction solvent, bakuchiol, the least polar compound among these three marker substances, was only slightly soluble.

IV. *Calibration Curves*

According to the statements in the experimental section, the calibration curves are shown in Table 1. The results showed good linear relationship between the corresponding peak-area ratio and concentration. All calibration curves were linear with correlation coefficients of 0.9995~0.9999. As shown in Table 1, the limits of detection for bakuchiol, psoralen and angelicin were 175.0, 37.5, and 43.7 ng/mL, respectively.

V. *Suitability Test*

To check the precision of the method, stock solutions containing various concentrations were injected three times on the same day (intraday) or on three consecutive days (interday). The relative standard deviations (RSD) are shown in Table 2. The RSD for intraday and interday were 0.3~1.5% and 0.2~1.0%, respectively. These data showed good accuracy with this HPLC method.

Table 2. The intra-day and inter-day analytical precision of bakuchiol, psoralen and angelicin

Compound	Concentration (μg/mL)	Intra-day (R.S.D. %) (n = 3)	Inter-day (R.S.D. %) (n = 3)
Bakuchiol	17.5	0.3	1.0
	35.0	1.1	0.3
	175.0	1.5	0.2
Psoralen	1.7	1.1	0.5
	14.5	0.8	0.6
	116.7	0.5	0.7
Angelicin	1.5	0.8	0.4
	12.5	0.6	0.6
	100.0	0.6	0.3

Table 3. Recoveries of bakuchiol, psoralen and angelicin from Fructus Psoraleae

Compound	Added (mg/g Buguzhi)	Recovery (%)	Average recovery (%)	R.S.D. (%)
Bakuchiol	17.5	97.6	101.6	3.5
	35.0	102.7		
	70.0	104.5		
Psoralen	5.0	99.7	98.7	1.1
	10.0	98.9		
	20.0	97.5		
Angelicin	5.0	100.0	100.8	0.6
	10.0	101.0		
	20.0	101.4		

Table 4. Contents of bakuchiol, psoralen and angelicin in Fructus Psoraleae

Sample[a]	Bakuchiol (mg/g)	Psoralen (mg/g)	Angelicin (mg/g)
CD-1	71.0 ± 2.0[b]	2.5 ± 0.1	2.3 ± 0.1
CD-2	59.5 ± 0.9	2.5 ± 0.0	2.2 ± 0.1
CD-3	57.5 ± 1.3	3.1 ± 0.1	2.8 ± 0.2
CD-4	54.8 ± 0.9	3.8 ± 0.1	3.5 ± 0.0
CD-5	48.4 ± 0.3	4.2 ± 0.1	3.4 ± 0.1
CD-6	47.5 ± 0.8	9.7 ± 0.3	8.9 ± 0.2
CD-7	47.5 ± 1.7	9.8 ± 0.2	8.8 ± 0.2
CD-8	46.5 ± 0.8	8.3 ± 0.3	7.1 ± 0.2
CD-9	39.7 ± 0.2	6.5 ± 0.2	6.0 ± 0.0
CD-10	36.2 ± 0.6	13.0 ± 0.6	9.2 ± 0.4

[a]CD-1~10 represent ten crude drugs purchased form different stores.
[b]Values are presented as means ± S.D., n = 3.

Pulverized Fructus Psoraleae spiked with three different amounts of stock solutions were analyzed to determine the recoveries of these three marker substances. As shown in Table 3, the average recoveries of bakuchiol, psoralen and angelicin were 101.6%, 98.7% and 100.8% with variation coefficients of 3.5%, 1.1%, and 0.6%, respectively.

VI. *Determination of Three Marker Substances in Crude Drugs and Commercial Concentrated Products of Fructus Psoraleae*

Ten crude drugs and eight commercial concentrated products of Fructus Psoraleae were collected and analyzed to determine the content of the above three marker substances. Bakuchiol, psoralen and angelicin contents were in the range of 36.2~71.0, 2.5~13.0 and 2.2~9.2 mg/g for crude drugs of Fructus Psoraleae, respectively (Table 4). The values are markedly different from each other, probably due to the variation of the source of the crude drugs and different processing methods.

The contents of bakuchiol, psoralen or angelicin ranged among 0.6~21.1, 0.6~5.2 and 0.6~5.3 mg/g for commercial concentrated products of Fructus Psoraleae (Table 5). The results showed that contents of these three active compounds in the commercial concentrated products were markedly different from each other, which could be due to different sources of the crude drugs or different manufacturing processes.

In conclusion, a HPLC method was developed for the simultaneous quantification of bakuchiol, psoralen, and angelicin in Fructus Psoraleae. The analysis of each sample can be completed within 30 min. This method possesses the advantages of simplicity, rapidity, high

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Table 5. Contents of bakuchiol, psoralen and angelicin in commercial concentrated products of Fructus Psoraleae

Sample[a]	Bakuchiol (mg/g)	Psoralen (mg/g)	Angelicin (mg/g)
CDP-1	0.6 ± 0.0[b]	0.6 ± 0.0	0.6 ± 0.0
CDP-2	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
CDP-3	1.8 ± 0.0	1.2 ± 0.0	1.1 ± 0.0
CDP-4	19.0 ± 0.6	5.0 ± 0.1	4.7 ± 0.1
CDP-5	11.8 ± 0.1	2.5 ± 0.0	2.4 ± 0.0
CDP-6	2.5 ± 0.0	3.2 ± 0.0	2.7 ± 0.0
CDP-7	21.1 ± 0.3	5.2 ± 0.0	5.3 ± 0.1
CDP-8	19.1 ± 0.3	3.5 ± 0.0	3.5 ± 0.1

[a]CDP-1~8 represent eight commercial concentrated products of Fructus Psoraleae purchased form eight different factories.
[b]Values are presented as means ± S.D., n = 3.

sensitivity and good reproducibility and will be applicable to the quality control of Fructus Psoraleae.

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REFERENCES

1. Chang, H. M. and But, P. P. H. 1986. Pharmacology and application of Chinese materia medica. pp. 634-641. World Scientific. Singapore.
2. Lin, Y. L. and Kao, Y. H. 1992. Two new benzofuran derivatives, corylifonol and isocorylifonol from the seeds of *Psoralea corylifolia*. Heterocyles 34: 1555-1564.
3. Mehta, G., Nayak, U. R. and Dev, S. 1966. Bakuchiol, a novel monoterpenoid. Tetrahedron Letters 38: 4561-4567.
4. Park, E. J., Zhao, Y. Z., Kim, Y. C. and Sohn, D. H. 2005. Protective effect of (s)-bakuchiol from *Psoralea corylifolia* on rat liver injury *in vitro* and *in vivo*. Planta Med. 71: 508-513.
5. Sun, N. J., Woo, S. H., Cassady, J. M. and Snapke, R. M. 1998. DNA polymerase and topoisomerase II inhibitors from *Psoralea corylifolia*. J. Nat. Prod. 61: 362-366.
6. Latha, P. G. and Panikkar, K. R. 1999. Inhibition of chemical carcinogenesis by *Psoralea corylifolia* seeds. J. Ethnopharmacol. 68: 295-298.
7. Adhikari, S., Joshi, R., Patro, B. S., Ghanty, T. K., Chintalwar, G. J., Sharma, A., Chattopadhyay, S. and Mukherjee, T. 2003. Antioxidant activity of bakuchiol: experimental evidences and theoretical treatments on the possible involvement of the terpenoid chain. Chem. Res. Toxicol. 16: 1062-1069.
8. Khatune, N. A., Islam, M. E., Haque, M. E., Khondkar, P. and Rahman M. M. 2004. Antibacterial compounds from the seeds of *Psoralea corylifolia*. Fitoterapia 75: 228-230.
9. Boyer, V., Moustacchi, E. and Sage, E. 1988. Sequence specificity in photoreaction of various psoralen derivatives with DNA: role in biological activity. Biochemistry 27: 3011-3018.
10. Liu, R., Li, A., Sun, A. and Kong, L. 2004. Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. J. Chromatogr. A 1057: 225-228.
11. Qiao, C. F., Han, Q. B., Song, J. Z., Mo, S. F., Kong, L. D., Kung, H. F. and Xu, H. X. 2006. Quality assesment of Fructus Psoraleae. Chem. Pharm. Bull. 54: 887-890.
12. Zhao, L., Huang, C., Shan, Z., Xiang, B. and Mei, L. 2005. Fingerprint analysis of *Psoralea corylifolia* L. by HPLC and LC-MS. J. Chromatogr. B 821: 67-74.
13. Chen, X., Kong, L., Su, X., Pan, C., Ye, M. and Zou, H. 2005. Integration of ion-exchange chromatography fraction with reversed-phase liquid chromatography-atmospheric pressure chemical ionization mass spectrometry for isolation and identification of compounds in *Psoralea corylifolia*. J. Chromatogr. A 1089: 87-100.
14. Wang, X., Wang, Y., Yuan, J., Sun, Q., Liu, J. and Zheng, C. 2004. An efficient new method for extraction, separation and purification of psoralen and isopsoralen from Fructus Psoraleae by supercritical fluid extraction and high-speed counter-current chromatography. J. Chromatogr. A 1055: 135-140.
15. Tsai, T. W. and Wang, E. C. 2004. A new synthesis of angelicin from 7-hydroxycoumarin via *C*-propenation-*O*-vinylation and ring-closing metathesis. J. Chin. Chem. Soc. 51: 1019-1023.
16. Wu, T. S., Shi, L. S., Wang, J. J., Iou, S. C., Chang, H. C., Chen, Y. P., Kuo, Y. H., Chang, Y. L. and Teng, C. M. 2003. Cytotoxic and antiplatelet aggregation principles of *Ruta graveolens*. J. Chin. Chem. Soc. 50: 171-178.
17. Jiangning, G., Xinchu, W., Hou, W., Qinghua, L. and Kaishun, B. 2005. Antioxidants from a Chinese medicinal herb - *Psoralea corylifolia* L. Food Chem. 91: 287-292.
18. Labbe, C., Faini, F., Coll, J. and Connolly, J. D. 1996. Bakuchiol derivatives from the leaves of *Psoralea glandulosa*. Phytochemistry 42: 1299-1303.