韓藥作誌(Korean J. Medicinal Crop Sci.) 16(3) : 150 – 154 (2008)

유통생약 황금의 품질모니터링 연구

이아영* · 추병길* · 천진미** · 김홍준*** · 장 설** · 이혜원* · 김호경*†

*한국한의학연구원 한약자원연구부, **한국한의학연구원 한약품질검사팀, ***우석대학교한의과대학 방제학교실

Quality Monitoring of Distributed Herbal Medicine, Scutellariae Radix

A Yeong Lee*, Byung Kil Choo*, Jin Mi Chun, Hong Jun Kim***,
Seol Jang**, Hye Won Lee*, and Ho Kyoung Kim*†**

**Department of Herbal Resources Research, Korea Institute of Oriental Medicine, Daejeon, 305-811, Korea.*
***Herbal Quality Control Team, Korea Institute of Oriental Medicine, Daejeon, 305-811, Korea.*
****Dept. of Prescriptionology, College of Oriental Medicine, Woosuk University, 565-701 Jeollabuk-do, Korea*

ABSTRACT : This study was investigated that quality inspection of distributed Scutellariae Radix in Korea. To evaluate the quality of these herbal medicines, we carried out TLC pattern, foreign matter in purity, loss on drying, ash, acid-insoluble ash, oil content, dilute ethanol-soluble, water-soluble, ether-soluble extracts contents, quantitative analyses, residual SO_2, individual heavy metals and organochlorine pesticides. To measure contents of baicalin, baicalein and wogonin, we were quantitative analyzed using HPLC. The average contents of baicalin, baicalein and wogonin were detected by 13.28 (± 0.43)%, 1.17 (± 0.04)% and 0.40 (± 0.02)%, respectively. Each average contents As, Cd, Hg and Pb was 0.059 (± 0.054) ㎎ · kg^{-1}, 0.019 (± 0.010) ㎎ · kg^{-1}, 0.017 (± 0.057) ppm and 0.242 (± 0.084) ㎎ · kg^{-1}, respectively.

Key Words **:** Scutellariae radix, Quality inspection, HPLC, Baiclin, Baicalein, Wogonin

INTRODUCTION

Herbal medicines have a long history in medical practice and health care especially in some Asian and African countries. During the last two decades, herbal medicines have expanded globally and gained considerable attention because of low toxicity and good therapeutical performance. Unfortunately, the quantification and quality of safety and efficacy data about the herbal medicines are far from sufficiency to satisfy the regulation supporting the world widely uses of these medicines (Yu *et al.*, 2007). The evaluation of efficacy and safety for herbal medicines were a priority objective to ensure these quality and to protect consumers against possible health risks.

Scutellaria baicalensis (Huang Qin or Chiense skullcap) is a Chinese herbal medicine which has been widely used as an anti-inflammatory, anti-viral, anti-bacterial, anti-cancer compound (Kumagai *et al.*, 2007). 'Huang-qin' has been routinely used in the treatment of bronchitis, hepatitis, allergy, inflammation, arteriosclerosis (Zhang *et al.*, 2006), various ailments including fevers, ulcers, inflammation and cancers (Kim *et al.*, 2006).

Scutellaria baicalensis contains a variety of flavones, pheylethanoids, amino acids, sterols and essential oils. Its dried roots contain over 30 kinds of flavonoids such as baicalin, baicalein, wogonin, wogonin-7-O-glucuronide, oroxylin A, croxylin A-7-O-glucuronide, apigenin, chrysin, scutellarein, skullcapflavon I, II and 3,5,7-trihydroxy-2'-methoxyflavanone (Li *et al.*, 2004; Horvath *et al.*, 2005; Kim *et al.*, 1998; Kim *et al.*, 2006). Bacalin, wogonin, wogonin glucuronide are main components in Scutellariae Radix (Lee *et al.*, 1993).

Baicalin is the most abundant component, and has anti-allergic, anti-inflammatory, anti-HIV, anti-tumor, antioxidant and free radical scavenging and anti-SARS coronavirus effects. It is known to have an effect on multiple biological functions, including the ability to inhibit aldose reductase activity and nitric oxide (NO) producing (Li *et al.*, 2004; Lim *et al.*, 2007; Li & Chen, 2005; Kim *et*

†Corresponding author: (Phone) +82-42-868-9502 (E-mail) hkkim@kiom.re.kr
Received October 1, 2007 / Revised March 24, 2008 / Accepted April, 12, 2008

al., 2006). Baicalein possesses anti-HIV, anti-tumor, antioxidant and free radical scavenging effects. Wogonin has anti-respiratory syncytial virus, anti-hepatitis B virus, anti-tumor, antioxidant and free radical scavenging effect (Li *et al.*, 2004; Li & Chen, 2005).

Previous separation methods of flavonoids in *S. baicalensis* Georgi have included capillary electrophoresis, thin-layer chromatography, gas chromatography and column chromatography (Horvath *et al.*, 2005), micellar electrokinetic capillary chromatography (MEKC) (Li *et al.*, 2004), high-speed counter-current chromatography (HSCCC). Chinese scientist developed another important technique, high-speed counter-current chromatography (HSCCC), based on liquid-liquid partition chromatography (Zgórka, 2006). Reverse-phase high-performance liquid chromatography (HPLC) has been the most widely used method of flavonoid analysis because of the polarity and low volatility of flavonoids (Horvath *et al.*, 2005).

The purpose of this study was tried to the simultaneous determination of distributed Scutellariae Radix compounds, *i.e.*, baicalin, baicalein and wogonin by HPLC. Monitoring of hazard materials for distributed herbal medicines were experimented on residual SO_2, individual heavy metals (As, Cd, Hg, Pb) and residual pesticides. The experiments were according to decree of Korea Food & Drug Administration (KFDA) 2005-44, 2005-62 and 2005-72, respectively.

MATERIALS AND METHODS

1. Plant materials

16 samples of commercial Scutellariae Radix were collected in Seoul, Daegu, Gwangju and Jecheon, Korea and identified by H. J. Kim in Woosuk University. A voucher specimen (No. 20005-03) of this materials has been deposited at the quality control of herbal medicine department of Korea Institute of Oriental Medicine (KIOM), Korea.

2. Reagents and instruments

Baicalin, baicalein, wogonin were purchased from Wako (Japan) of HPLC grades. Methanol was obtained from J. T. Baker (USA) of HPLC grade. TLC plate was used by Merck (Germany). HPLC was measured with a Shimadzu LC-10A*vp* system (Japan). GC/MS and GC/ECD were used Shimadzu QP-5050A series and GC-10A, respectively. ICP and Mercury analyser were measured with Thermo Jarrell Ash Co., and Nippon Instrumen Corporation, respectively. Drying oven, Maffle's furnace and ultrasonic bath were carried out on a FF-YG-50 of Korea electronics (Korea), F48015 of Branstead Thermolyne (USA) and 8210 of Branson (USA), respectively.

3. Identify, purity, loss on drying, ash, acid-insoluble ash, extract contents

We were conformed to monographs, part II of The Korean Pharmacopoeia (KP) methods.

4. Residual SO_2, individual heavy metals and residual pesticides

We were followed by decree of Korea Food & Drug Administration KFDA 2005-44, 2005-62 and 2005-72.

5. Standard stock solution

Standards of baicalin, baicalein and wogonin were dissolved in methanol to make each stock solution of which concentration were 0.642 ㎎ · ㎖$^{-1}$, 0.159 ㎎ · ㎖$^{-1}$ and 0.122 ㎎ · ㎖$^{-1}$, respectively. All the solutions were filtered through a 0.45 ㎛ membranes and degassed by an ultrasonic bath. Regressive equation (coefficient of correlation) for baicalin, baicalein and wogonin were $y = 32208.3756x + 215847.9583$ ($r^2 = 0.9982$), $y = 55579.0811x - 451855.333$ ($r^2 = 0.9993$) and $y = 61341.0210x + 11918.8333$ ($r^2 = 0.9998$), respectively.

6. Sample preparation

Accuracy 0.5 g of pulverized Scutellariae Radix samples were extracted 50 ㎖ of 75% acetonitrile in 0.2 M phosphoric acid mixture solution under 80℃ for 2 hours and filtered. Added 50 ㎖ of acetonitrile in 0.2 M phosphoric acid mixture solution to residue and proceeded in the same manner. After cooling, all the filtrates were added to make exactly 100 ㎖ acetonitrile in 0.2 M phosphoric acid mixture solution. All samples were finally filtered through a 0.45 ㎛ membrane and a 10 ㎕ portion of those solutions were injected into the HPLC system (Scheme 1). We were made a experiment 3 times above methods and calculated contents of baicalin, baicalein and wogonin through peak area and regressive equation.

7. HPLC analytical condition

Analyses were performed on a Luna C_{18} analytical column (4.6 × 250 ㎜ 5 ㎛, Phenomenex). Mobile phase was

a mixture of acetonitrile : 1.0% acetic acid in distilled water (70% (v/v, 8 min) → 65% (v/v, 15 min) → 50% (v/v, 40 min)) and flow rate was 1.0 mg · mℓ$^{-1}$. The analyses were monitored at 277 nm UV wavelength.

RESULTS AND DISCUSSION

1. Identify

Ethanol solution add 1~2 drop of diluted ferric chloride, after its solution showed green color and changed to purple-brown. Thin layer chromatography (TLC) pattern analysis of baicalin and wogonin were showed a point which equaled to standard point at R_f value of 0.38, 0.98 for all samples, respectively.

2. Purity

The amounts of foreign matter were contained average 1.08 (±0.51)%. All samples were measured 0.28~1.96% (Table 1).

3. Loss on drying, ash and acid-insoluble ash

Average loss on drying, ash and acid-insoluble ash of samples were measured to 10.73 (±1.71)%, 4.74 (±0.56)% and 0.59 (±0.15)%. In case of loss on drying, 1 sample of 16 samples exceeded the standard limit, *i. e.* 15.0% which were measured 8.61~15.63%. In case of ash and acid-insoluble ash, All samples were less than standard limit, *i. e.* 6.0% and 1.0%. The measuring range of those samples were showed 3.72~5.88%, 0.38~0.91%, respectively (Table 1).

4. Extract contents

Average contents of dilute ethanol-soluble, water-soluble and diethyl ether-soluble extracts were obtained to 50.81 (±6.09)%, 35.10 (±5.98)% and 1.49 (±1.18)%, respectively. Those of values were 37.85~58.30%, 26.30~39.38% and 0.47~5.01%, respectively (Table 1).

5. Contents of baicalin, baicalein and wogonin

Baicalin, baicalein and wogonin (Fig. 1) were analysed by C_{18} column because the polarity and low volatility of flavonoids (Horvath *et al.*, 2005). Retention times of the components were showed at about 10.7 min, 23.7 min and 36.3 min, respectively (Fig. 2). And contents of baicalin, baicalein and wogonin were detected on average 13.28 (±0.43)%, 1.17 (±0.04)% and 0.40 (±0.02)%, respectively.

Table 1. Experimental data of purity, loss on drying, ash, acid-insoluble ash, extract contents and contents of baicalin, baicalein and wogonin.

	Purity (%)	Loss on drying (%)	Ash (%)	Acid-insoluble ash(%)	Extract contents(g)			Contents (%)		
					Water	Diluted ethanol	Diethyl ether	Baicalin	Baicalein	Wogonin
Standard limit	-	Less than 15.0%	Less than 6.0%	Less than 1.0%	–	–	–	Sum of more than 10%		
1	1.35	12.40	4.66	0.84	26.70	42.12	2.42	15.56	1.17	0.45
2	0.92	11.20	4.35	0.61	44.53	50.25	1.16	12.65	0.62	0.29
3	0.75	15.63	3.72	0.50	46.28	51.71	1.10	9.24	2.62	0.94
4	0.46	12.50	4.24	0.64	36.85	49.80	1.56	14.41	1.39	0.48
5	1.09	9.85	4.12	0.52	32.77	45.63	1.93	13.83	0.62	0.23
6	1.39	9.58	4.45	0.66	35.85	50.71	1.54	13.48	1.18	0.28
7	1.96	9.62	4.43	0.58	37.57	54.83	5.01	12.32	0.84	0.20
8	0.74	9.72	4.39	0.67	35.84	58.22	1.72	13.93	1.80	0.41
9	1.74	9.09	5.14	0.45	27.57	47.99	0.70	13.53	0.88	0.24
10	1.07	11.35	5.13	0.46	36.43	56.89	1.36	13.47	1.03	0.36
11	1.35	10.27	5.26	0.55	27.16	43.85	0.87	14.18	1.94	0.73
12	1.49	8.61	5.51	0.44	32.76	52.73	0.47	11.51	1.06	0.32
13	0.28	9.72	4.63	0.75	38.20	57.66	0.91	16.61	0.78	0.25
14	0.46	10.25	4.91	0.38	39.38	58.30	3.55	13.37	0.98	0.46
15	0.54	11.21	5.88	0.91	26.34	37.85	3.8	10.76	0.87	0.34
16	1.66	10.75	5.03	0.45	37.37	54.36	3.07	13.64	1.00	0.34
Average	1.08	11.07	4.74	0.59	35.10	50.81	1.49	13.28	1.17	0.40
SD	0.51	1.71	0.56	0.15	5.98	6.09	1.18	0.43	0.04	0.02

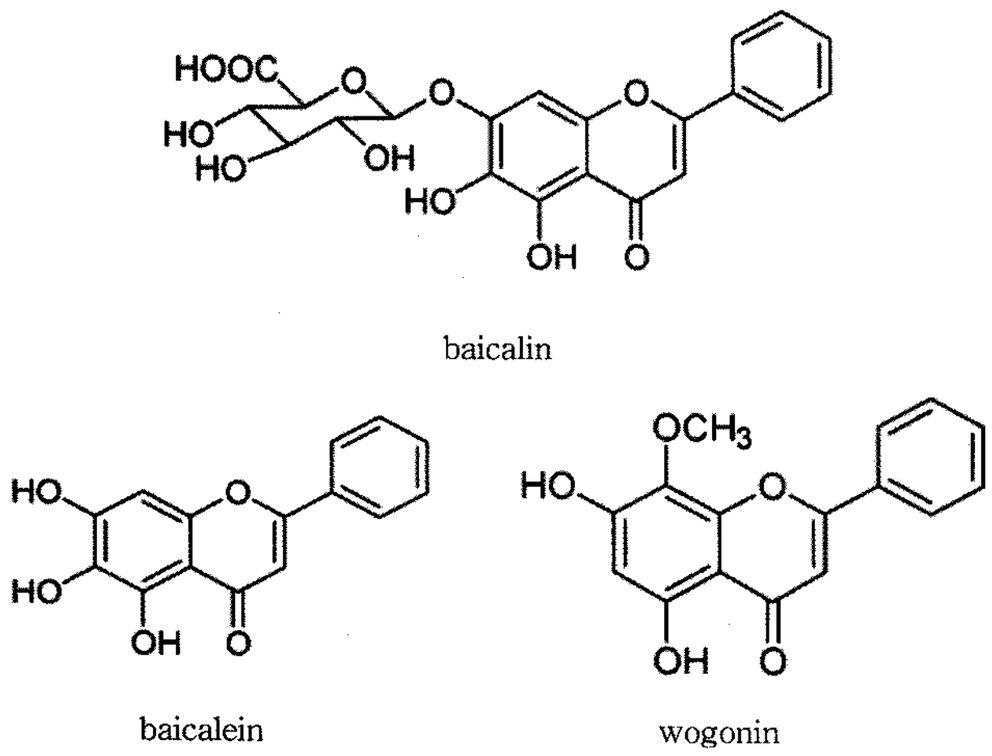

Fig. 1. Chemical structures of baicalin, baicalein and wogonin.

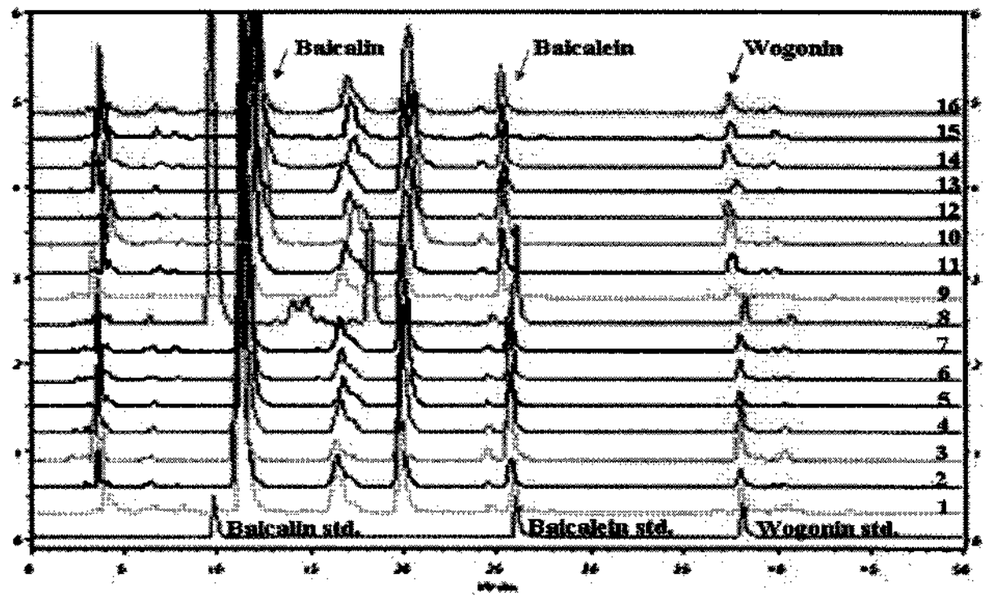

Fig. 2. HPLC chromatography of Scutellariae Radix according to the collected site.

1 sample of 16 samples was not exceed 10.0% which were measured 9.24~15.56% for baicalin. Each of average content of baicalein and wogonin for 16 samples was determined to 1.17 (± 0.04)%, 0.40 (± 0.02)%, respectively. Detected value ranges of three standards for all samples were 0.62~2.62% and 0.20~0.94%, respectively. As result of research, All of samples contents were more than 0.6% for baicalein and all of samples were more than 0.2% for wogonin. For previously research, average contents of baicalin, baicalein and wogonin were about 24.74~143.56 $mg \cdot g^{-1}$, 1.53~15.12 $mg \cdot g^{-1}$ and 0.36~4.80 $mg \cdot g^{-1}$ (Yu *et al.*, 2006). These components could be indicated and represented efficacy for Scutellariae Radix. Result of this study, sum of the contents for baicalin, baicalein and wogonin should be suitable for standard limit *i.e.* more than 10.0% (Table 1).

Table 2. Experimental data of residual SO_2, individual heavy metals and residual pesticides.

	Residual SO_2	Individual heavy metals				Residual pesticides				
		Pb	As	Cd	Hg	Total BHC	Total DDT	Aldrin	Endrin	Dieldrin
Standard limit	500 ppm	5.0 mg/kg	3.0 mg/kg	0.3 mg/kg	0.2 mg/kg	0.2 mg/kg	0.1 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
1	2.79	0.385	0.164	0.039	0.230	N. D.†	N. D.	N. D.	N. D.	N. D.
2	2.08	0.335	0.033	0.013	0.007	N. D.	N. D.	N. D.	N. D.	N. D.
3	2.71	0.322	0.021	0.029	0.004	N. D.	N. D.	N. D.	N. D.	N. D.
4	1.69	0.291	0.040	0.016	0.004	N. D.	N. D.	N. D.	N. D.	N. D.
5	0.70	0.368	0.021	0.016	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
6	147.73	0.258	0.037	0.016	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
7	121.07	0.124	0.021	0.013	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
8	3155.77	0.115	0.201	0.007	0.015	N. D.	N. D.	N. D.	N. D.	N. D.
9	2.74	0.264	0.020	0.014	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
10	217.34	0.148	0.027	0.018	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
11	2.76	0.237	0.062	0.040	0.001	N. D.	N. D.	N. D.	N. D.	N. D.
12	178.36	0.243	0.038	0.016	0.002	N. D.	N. D.	N. D.	N. D.	N. D.
13	1.74	0.149	0.063	0.007	0.001	N. D.	N. D.	N. D.	N. D.	N. D.
14	1.71	0.200	0.031	0.032	0.000	N. D.	N. D.	N. D.	N. D.	N. D.
15	1.76	0.226	0.120	0.011	0.001	N. D.	N. D.	N. D.	N. D.	N. D.
16	261.49	0.204	0.051	0.014	0.001	N. D.	N. D.	N. D.	N. D.	N. D.
Average	256.40	0.24	0.059	0.019	0.017	N. D.	N. D.	N. D.	N. D.	N. D.
SD	778.50	0.08	0.05	0.01	0.057					

†N. D.: Not Detected

6. Residual SO_2, individual heavy metals and residual pesticides

1 sample of 16 samples exceeded the standard limit, *i. e.* 500 mg · kg^{-1} which were measured 0.7~3155.77 mg · kg^{-1} for residual SO_2. In case of Pb, As and Cd, all of samples were less than standard limit, *i. e.* 5.0 mg · kg^{-1}, 3.0 mg · kg^{-1}, 0.3 mg · kg^{-1} for individual heavy metals. Detected value of Pb, As and Cd were 0.12~0.39 mg · kg^{-1}, 0.02~0.16 mg · kg^{-1} and 0.01~0.04 mg · kg^{-1}, respectively. But 1 sample of 16 samples was more than standard limit, *i. e.* 0.2 mg · kg^{-1}. The measuring range of Hg were detected 0.001~0.23 mg · kg^{-1} for individual heavy metals. All samples were not detected for total benzene hexachloride (BHC), total dichlorodiphenyltrichloroethane (DDT), aldrin, endrin and dieldrin. Standard limit of total BHC, DDT, aldrin, endrin and dieldrin were stipulated for 0.2 mg · kg^{-1}, 0.1 mg · kg^{-1}, 0.01 mg · kg^{-1}, 0.01 mg · kg^{-1} and 0.01 mg · kg^{-1}, respectively (Table 2). We should be continuous monitored for hazard components and make an effort decrement of these factors.

CONCLUSION

The result of quality inspection of distributed Scutellariae Radix, 1 sample was more than standard limit of loss on drying. All samples were in exceeded of 10% *i. e.* contents of sum for baicalin, baicalein and wogonin were more than 10%. The result of safety test, each one sample was not satisfied with residual SO_2 and content of Hg, respectively. For guarantee of efficacy and safety for herbal medicines, it is suggest that we should be continue to quality inspection.

ACKNOWLEDGEMENTS

This study was supported from Ministry of Health & Welfare in Korea and Korea Institute of Oriental Medicine (KIOM).

LITERATURE CITED

Horvath CR, Martos PA, Saxena PK (2005) Identification and quantification of eight flavones in root and shoot tissues of the medical plant Huang-qin (*Scutellaria baicalensis* Georgi) using high-performance liquid chromatography with diode array and mass spectrometric detection. Journal of Chromatography A 1062:199-207.

Kim DH, Kim HK, Park SJ, Kim JY, Zou Y, Cho KH, Kim YS, Kim DH, Yu BP, Choi JS, Chung HY (2006) Shot-term feeding of baicalin inhibits age-associated NF-κB activation. Mechanism of Ageing and Development 127(9):719-725.

Kim MS, Chung BJ, Park GC, Park TD, Kim SC, Shim JH (1998) Effects of topping time and split fertilization on growth and root yield of *Scutellaria baicalensis* G. Korean J. Medicinal Crop Sci. 6(4):271-276.

Kim SC, Ahn KS, Park CK, Jeon BS, Lee JT, Park WJ (2006) Isolation of antioxidative compound from *Scutellaria baicalensis* G. Korean J. Medicinal Crop Sci. 14(4):212-216.

Kim YH, Jeong DW, Paek IB, Ji HY, Kim YC, Sohn DH, Lee HS (2006) Liquid chromatography with tandem mass spectrometry for the simultaneous determination of baicalein, baicalin, oroxylin A and wogonin in rat plasma. Journal of Chromatography B 844(2):261-267.

Kumagai T, Müller CI, Desmond JC, Imai Y, Heber DH, Koeffler P (2007) *Scutellaria baicalensis*, a herbal medicine: Anti-proliferative and apoptotic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines. Leukemia Research 31(4):523-530.

Lee MS, Kim KH, O KH (1993) Plant regeneration from leaf tissue culture and some effective substances in *Scutellaria baicalensis* G. Korean J. Medicinal Crop Sci. 1(1):43-48.

Li HB, Chen F (2005) Isolation and purification of baicalein, wogonin and oroxylin A from the medical plant *Scutellaria baicalensis* by high-speed counter-current chromatography. Journal of Chromatography A 1074:107-110.

Li HB, Jiang Y, Chen F (2004) Separation methods used for *Scutellaria baicalensis* active components. Journal of Chromatography B 812:277-290.

Lim BO, Choi SY, Choi DK, Park PJ, Choi WS, Kim JD, Shin HM (2007) Inhibitory effect of Scutellaria baicalensis root extract on chemical mediator release and immune response. Korean J. Medicinal Crop Sci. 15(2):132-137

Yu K, Gong Y, Lin Z, Cheng Y (2007) Quantitative analysis and chromatographic fingerprinting for the quality evaluation of *Scutellaria baicalensis* Georgi using capillary electrophoresis. Journal of Pharmaceutical and Biomedical Analysis 43(2):540-548.

Zgórka G (2006) Retention behavior of silica-bonded and novel polymeric reversed-phase sorbents in studies on flavones as chemotaxonomic markers of *Scutellaria* L. genus. Journal of Chromatography A 1120:230-236.

Zhang Y, Wang X, Wang X, Xu Z, Liu Z, Ni Q, Chu X, Qiu M, Zhao A, Jia W (2006) Protective effect of flavonoids from *Scutellaria baicalensis* Georgi on cerebral ischemia injury. Journal of Ethnopharmacology 108(3):355-360.