

Original Article

Quality Monitoring of Distributed *Schizandra chinensis* (Turcz.) Baill in Korea

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Object : This study investigated the quality inspection of *Schizandra chinensis* (Turcz.) Baill distributed in Korea.
Methods: To evaluate the quality of these herbal medicines, we carried out TLC pattern analysis, purity, loss on drying, ash, acid-insoluble ash, essential oil contents, dilute ethanol-soluble, water-soluble, ether-soluble extracts and HPLC analysis.
Results : As a result, TLC pattern analysis of schizandrin and gomisin A showed $R_f=0.64$ and 0.74 , respectively. To measured content of schizandrin and gomisin A, we quantitatively analyzed using HPLC. The average contents of schizandrin and gomisin A were detected to be $0.60 (\pm 0.02)\%$ and $0.12 (\pm 0.004)\%$, respectively.
Discussion : As a result of this study, we suggest that the minimum content of schizandrin and gomisin A should be 0.5% and 0.1% , respectively. We could suggest that the minimum of essential oil content should be 0.6 ml .

Key Words : *Schizandra chinensis* (Turcz.) Baill, monitoring, schizandrin, gomisin A

Introduction

Schizandra chinensis (Turcz.) Baill, a popular traditional medicine, grows wild in the most Eastern parts of Korea, Japan, China, and Russia¹⁾. There are 25 species of the *schizandra* genus worldwide, with most found in China²⁾. *Schizandra* fruit is a sap fruit of irregular sphere or spheroid shape, about 6 mm in diameter. The external surface is dark red to blackish brown, with wrinkles and occasionally with white powder³⁾. Fruits and seeds contain mainly dibenzo-[a,

c,]-cyclooctadiene lignans such as schizandrin, gomisin A, gomisin C, deoxyschizandrin and γ -schizandrin, epigalbacin, volatile oil containing monoterpenes, sesquiterpene, (+)- α -ylangene, chamigrenal and so on^{1,4-9)}.

Schizandra chinensis has been utilized in traditional medicine as an anti-aging, antitussive, antihepatotoxic, antiasthmatic, antidiabetic, sedative, and tonic agent¹⁰⁻¹¹⁾. Some dibenzo[a, c]cyclooctadiene lignans were identified as a potent anti-human immunodeficiency virus agent¹²⁾, prevented liver injuries, inhibited lipid peroxidation⁵⁾, and were observed to have antimicrobial¹³⁾ and antioxidative activity¹⁴⁾ effects. The effects of deoxyschizandrin on alanine transaminase, aspartate aminotransferase, albumin and total protein serum show that deoxyschizandrin can afford protection against CCl₄ induced hepatic damage^{4,15)}. Schizandrin B could protect against myocardial ischemia-reperfusion

Received 10 December 2006; received in revised form 11 December 2006; accepted 12 December 2006
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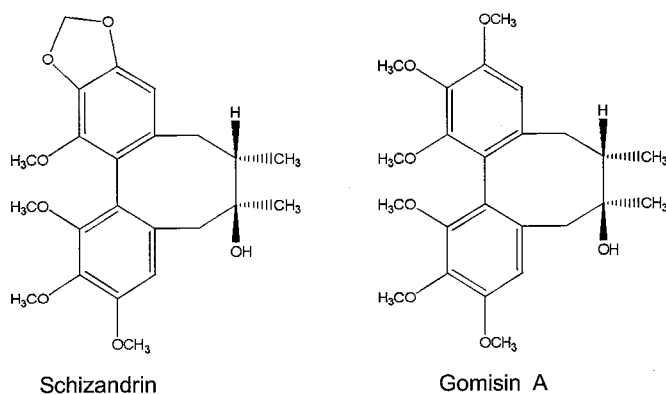


Fig. 1. Chemical structures of shizandrin and gomisin A.

injury and chemically induced liver injury in rodents. The cardio- and hepato-protective action of schizandrin B was believed to be related to its *in vivo* antioxidative potential¹⁶). Recently *S. chinensis* has become a raw material for the preparation of nutraceuticals used by sportsmen for nutrition and as a preventive means against civilization diseases of the cardiovascular system⁶).

Previously described methods for the determinations of the lignans in *Schizandra chinensis* species plants include TLC, capillary GC-FID, GC-MS, HPLC (6, 12, 17-19), LC-ES-MS²), capillary electrochromatography (CEC)⁵), and high-speed counter-current chromatography (HSCCC)^{1,4}) techniques.

Current quality control systems for distributed herbal medicines have raised several points,

especially distribution of low quality and inferior herbal medicines. In spite of oil contents, schizandrin and gomisin A were main constituents for *Schizandra chinensis*, yet assay method was not listed in KP. So we suggested advanced quality specification of *Schizandra chinensis* by chemistry methods, and suggest that minimums of oil contents, schizandrin and gomisin A could be standardized by monitoring.

Materials and Methods

Plant materials. 16 samples of distributed *Schizandra chinensis* were collected in Seoul, Daegu, Gwangju and Jecheon, Korea and identified by H. J. Kim at the Korea Institute of Oriental Medicine (KIOM). A voucher specimen

Schizandra chinensis 2.0 g	
	Reflux with 50 ml methanol 2 hrs (x2)
Methanol extract	
	Filtered with 0.45 μm membrane filter Adjust 100 ml with MeOH
Sample solution 10 μl for HPLC analysis (Methanol : acetonitrile : water = 11 : 11 : 8 (v/v) at 220 nm)	

Scheme 1. Preparation of test materials for quantitative analysis of schizandrin and gomisin A in *Schizandra chinensis*

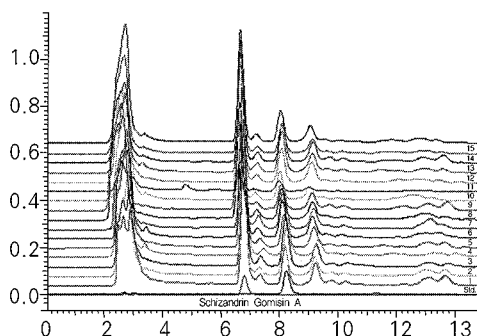


Fig. 2. HPLC chromatography of *Schizandra chinensis* according to the collected site.

(No. 20005-Schi-01) of this material has been deposited at the quality control of herbal medicine department of KIOM, Korea.

Reagents and instruments. Schizandrin and gomisin A of HPLC grades (Fig. 1) were purchased from Wako and ChromaDex (USA), respectively. Methanol of HPLC grade was obtained from J. T. Baker (USA). TLC plate used was by Merck (Germany). HPLC was measured with a Shimadzu LC-10Avp system (Japan). Drying oven, muffle furnace and ultrasonic bath were carried out on an FF-YG-50 of Korea electronics (Korea), F48015 of Barnstead Thermolyne (USA) and 8210 of Branson (USA), respectively.

Identification. Silica gel TLC pattern analysis was performed by methanol extraction with mixture of ethyl acetate, hexane and acetic acid (10:10:1)³⁾.

Purity. The amount of receptacle, peduncle and other foreign matter selected from *Schizandra* fruit³⁾.

Loss on drying. 2~6 g of powder for *Schizandra chinensis* dried at 105°C for 5 hours, allow to cool in a desiccator and weighed accurately³⁾.

Ash. 2~4 g of powder for *Schizandra chinensis* heated between 500~550°C, ignited to incinerate the residue for more than 4 hours until no

carbonized substance remained³⁾.

Acid-insoluble ash. 25 ml of dilute HCl added to the total ash and boiled for 5 minutes³⁾.

Dilute ethanol-soluble extract. 70 ml dilute ethanol added to 2.3 g of the samples shaken for about 1 day³⁾.

Water-soluble extract. 70 ml water added to 2.3 g of the samples shaken for about 1 day³⁾.

Diethyl ether-soluble extract. 70 ml diethyl ether added to 2.3 g of the samples refluxed for about 4 hours³⁾.

Essential oil content. 500 ml water added to 50 g of the samples refluxed about 5 hours at 130~150°C³⁾.

Standard stock solution. Standards of schizandrin and gomisin A were dissolved in methanol to make each stock solution of which concentrations were 0.136 mg/ml and 0.114 mg/ml, respectively. All the solutions were filtered through a 0.45 µm membrane and degassed by an ultrasonic bath. Regressive equation (coefficient of correlation) for schizandrin and gomisin A were $y=60070.8747x + 84826.7391$ ($r^2=0.9999$) and $y=92678.3060x + 113886.5217$ ($r^2=1.0000$), respectively.

Sample preparation. Exactly 2.0 g of pulverized *Schizandra chinensis* samples were extracted in 50 ml of methanol under 80°C for 2 hours and

Table 1. Experimental data of purity, loss on drying, ash, acid-insoluble ash, extract contents; essential oil content and contents of schizandrin and gomisin A.

Item No.	Purity (%)	Loss on drying (%)	Ash (%)	Acid-insoluble ash (%)	Extract contents (%)			Essential oil (ml)	Contents of Standards (%)	
					diluted ethanol	water	diethyl ether		Schizandrin	Gomisin A
KP	less than 1.0%	-	less than 5.0%	-	-	-	-	-	-	-
1 (Korea)	2.43	11.39	3.44	0.33	37.16	37.34	11.94	0.60	0.65	0.13
2 (Korea)	1.61	11.61	4.14	0.47	39.77	37.12	12.79	0.70	0.60	0.11
3 (Korea)	1.91	10.69	3.85	0.44	26.94	25.70	16.87	0.73	0.74	0.18
4 (Korea)	0.14	10.65	4.98	0.71	37.74	42.59	12.10	0.70	0.51	0.11
5 (Korea)	0.59	12.04	3.80	0.41	34.88	37.12	12.77	0.70	0.63	0.11
6 (Korea)	0.35	11.14	4.90	0.75	42.32	43.35	16.16	0.73	0.74	0.12
7 (Korea)	0.71	13.37	4.70	0.72	40.94	42.40	16.67	0.73	0.56	0.15
8 (Korea)	1.41	11.08	4.64	0.73	48.09	45.27	12.94	0.73	0.61	0.11
9 (Korea)	0.71	12.66	2.55	0.35	38.36	38.52	8.53	0.60	0.45	0.09
10 (Korea)	1.56	11.06	4.39	0.46	42.31	36.53	8.96	0.63	0.44	0.09
11 (Korea)	1.82	14.09	3.13	0.37	35.61	40.49	9.03	0.80	0.66	0.16
12 (Korea)	0.20	15.70	4.63	0.59	29.80	41.78	10.23	0.70	0.73	0.13
13 (Korea)	1.20	15.09	4.56	0.65	31.64	36.20	7.89	0.60	0.56	0.14
14 (Korea)	0.86	14.52	4.44	0.54	38.68	42.18	8.91	0.73	0.65	0.14
15 (Korea)	0.75	13.84	3.87	0.46	47.34	45.77	13.90	0.80	0.56	0.10
16 (Korea)	4.51	12.52	3.68	0.42	44.78	43.37	12.28	0.77	0.62	0.12
Average	1.30	12.59	4.08	0.53	38.53	39.72	12.00	0.70	0.60	0.12
SD	1.08	1.65	0.67	0.15	5.92	4.91	1.65	0.07	0.02	0.004

filtered. 50 ml of methanol was added to the residue and the process repeated. After cooling, all the filtrates were added to make exactly 100 ml methanol. All samples were finally filtered through a 0.45 μm membrane and a 10 μl portion of those solutions were injected into the HPLC system (Scheme 1). We repeated the experiment 3 times in total using these methods and calculated contents of schizandrin and gomisin A through peak area and regressive equation.

HPLC analytical condition. Analyses were performed on a Luna C18 analytical column (4.6 \times 250 mm 5 μm , Phenomenex). Mobile phase was a mixture of methanol-acetonitrile-distilled water (11:11:8, v/v) and flow rate was 1.0 mg/ml -1. The analyses were monitored at 220 nm UV

wavelength.

Results and Discussion

1. Identification

Under the UV 254 nm wavelength, schizandrin and gomisin A showed a point which equaled the standard point at Rf value of 0.64 and 0.74 for all samples, respectively. Recently an identity test was established only for schizandrin by KP (8th edition), so we experimented not only for schizandrin but also gomisin A for distributed *Schizandra chinensis*.

2. Purity

The amounts of receptacle, peduncle and other

foreign matter contained averaged 1.30 (± 1.08)%. 8 samples of 16 samples exceeded the standard limit, i.e. 1.0% which were measured 1.20~4.51% (Table 1). Specially, some of the samples showed living insect and rotten fruits.

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

Average loss on drying, ash and acid-insoluble ash of samples were measured to 12.59 (± 1.65)%, 4.08 (± 0.67)% and 0.53 (± 0.15)%. The measuring range of those samples showed 10.65~15.70%, 2.55~4.98% and 0.33~0.75%, respectively (Table 1).

4. Extract contents

Average contents of dilute ethanol-soluble, water-soluble and diethyl ether-soluble extracts were obtained to 38.53 (± 5.92)%, 39.72 (± 4.91)% and 12.00 (± 1.65)%. Those values were 26.94~48.09%, 25.70~45.77% and 7.89~16.87%, respectively (Table 1).

5. Essential oil content

Average value of essential oil contents for all samples was 0.70 (± 0.07) ml and measured values of those samples were 0.60~0.80 ml. From previous research, essential oil consisted of fifty six components which represented about 98% of the total content²⁰. Yield of distillation extraction and simultaneous distillation extraction methods were obtained to each of 0.64% and 0.88%²¹. We suggest that the minimum of essential oil content should be 0.6 ml (Table 1).

6. Contents of schizandrin and gomisin A.

Schizandrin and gomisin A were chromatographed in order to determine their retention time at about 6.85 min and 8.53 min, respectively

(Fig. 2). Each of average contents of two standards for 16 samples was determined to 0.60 (± 0.02)% and 0.12 (± 0.004)%. Detected value ranges of two standards for all samples were 0.44~0.74% and 0.09~0.18%, respectively. As a result of our measurement, 2 of the samples' contents were not more than 0.5% for schizandrin and 2 were less than 0.1% for gomisin A. From previous research, average contents of schizandrin and gomisin A were about 0.7% and 0.2% (9, 18). As a result of this study, we suggest that the minimum content of schizandrin and gomisin A should be 0.5% and 0.1%, respectively (Table 1).

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