—Original— Natural Medicines 54 (5), 213-218 (2000)

Chemical Comparison of Astragali Radix (Huangqi) From Different Regions of China

X. Q. Ma[1,3], J. A. Duan[2], D. Y. Zhu[3], T. X. Dong[1] and K. W. K. Tsim[1] *

[1] *Department of Biology and Biotechnology Research Institute, The Hong Kong University of Science and Technology, Clear Water Bay Road, Hong Kong, China*

[2] *Jiangsu Research Institute of Traditional Chinese Medicine, Maigaoqiao, Nanjing 210028, China*

[3] *State Key Laboratory for New Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China*

(Received April 11, 2000)

Astragali Radix (Huangqi; 黄芪) is a popular traditional Chinese medicine for Qi-deficiency and Yang-weakness. The active constituents include saponins, flavonoids and polysaccharides; however, the levels of these components vary in different sources of Astragali Radix. By using HPLC and spectrophotometry, the amounts of isoflavonoids I-V, astragalosides I-IV and total polysaccharide were determined in two Astragali Radices: *Astragalus membranaceus* and *A. membranaceus var. mongolicus*; its adulterants: *A. hoantchy*, *A. lehmannianus*, *A. aksuensis* and *A. propinquus*; and its substitute: *Hedysarum polybotrys* (Hedysari Radix, Hongqi). Various specimens of these plants, either cultivated or wild, from different regions of China were also analyzed for their saponin, isoflavonoid and polysaccharide contents. The results showed that the amounts of isoflavonoids I-V and astragalosides I-IV varied in different species, and in plants from different habitat regions. The chemical composition was compared between various species sources of Astragali Radix, and their quality was discussed to give useful information for the quality control of Astragali Radix.

Keywords---astragalosides; isoflavonoids; Astragali Radix; Huangqi; Hedysari Radix; HPLC

In traditional Chinese medication, when one has Qi-deficiency and Yang-weakness one will be prescribed to take Astragali Radix (Huangqi; 黄芪) that has been proved to be an immunostimulant, tonic (adaptogenic), hepatoprotective, diuretic, antidiabetic, analgesic, expectorant and sedative drug[1,2,3,4]. Although Astragali Radix has a long history of medicinal use in Chinese herbal medicine, its pharmacological properties and clinical applications have not been studied until recently. Astragali Radix has been demonstrated to have a wide range of immunopotentiating effects, and used as an adjunct medicine during cancer therapy[4]. Demand for Astragali Radix is enormous through out the world, particularly in the market of South East Asia and Japan. The genus *Astragalus* L. comprises of 278 species, 2 subspecies, 35 varieties and 2 forma in China[5], of which, twelve carry the name Huangqi in the market, and are cultivated in more than 10 different regions of China. The morphological appearances of Huangqi and its adulterants show a great resemblance. Definite identification and quality control of Huangqi, therefore, are very difficult. The most commonly used Huangqi are radices of *Astragalus membranaceus* (Fischer) Bunge and *Astragalus membranaceus* (Fisch.) Bge. *var.*

mongholicus (Bge.) Hsiao[6], both of which are authentic botanical sources of Huangqi in Chinese Pharmacopoeia[1,2]. *Astragalus membranaceus* (Fisch.) Bge *var. mongholicus* (Bge.) Hsiao is often called *Astragalus mongholicus* Bunge in Japan. Hongqi (Hedysari Radix) is the root of *Hedysarum polybotrys* Handel-Mazzetti which is closely related to the authentic *Astragalus* plant, and could be used as a substitute of Huangqi. In addition, *Astragalus hoantchy* Franch., *Astragalus lehmannianus* Bunge, *Astragalus aksuensis* Bunge and *Astragalus propinquus* Schischk are common adulterants of Huangqi.

Of the active constituents in Astragali Radix, i. e. saponins, isoflavonoids, polysaccharides, γ-aminobutyric acid and various trace elements[4]. Astragaloside IV is used as a marker for the quality control of Huangqi. The levels of these active constituents in Astragali Radix determined by the TLC scanning and colorimetry method were shown to vary according to their origin[4,7]. Today, Astragali Radix is mostly prepared from cultivated plants. For example, *A. membranaceus* and *A. membranaceus var. mongholicus* are cultivated mainly in the north part (Shanxi, Neimengu, Hebei) and the north-east part (Heilongjiang) of China. Which are the best regions in China to produce good quality Huangqi? In the present study, in order to compare the quality of Astragali Radix from different regions of China, the levels of astragalosides I-IV, isoflavonoids I-V and polysaccharide in different Astragali Radices were determined by reverse phase high-performance liquid chromatography (HPLC) and spectrophotometry.

MATERIALS AND METHODS

Plant materials The roots and aerial parts of *A. membranaceus*, *A. membranaceus var. mongolicus*, *A. hoantchy*, *A. lehmannianus*, *A. aksuensis*, *A. propinquus* and *H. polybotrys* were collected from different regions of China as listed in Table 2. They were collected in August and September of 1999. Their corresponding voucher specimens were deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai China. The materials for analysis were finely powdered and kept dry until use.

Extraction of isoflavonoids and saponins 5 g of ground powder was extracted three times in a soxhlet with 100 ml aq. MeOH (MeOH/H_2O=4/1) for 2 hour. The combined MeOH extract was filtered, and evaporated to dryness in vacuo. For isoflavonoid assay, the viscous residue was stirred in 25 ml hot water, and the suspension was treated with 10 ml, 7.5 ml and then 5 ml of ethyl acetate. The aqueous lower phase containing sugar was discarded. The combined ethyl acetate phase containing isoflavonoids ~12.5 ml was evaporated to dryness in vacuo. Saponins were analogously extracted by using *n*-butanol saturated with water, and the butanol extract was concentrated. Both viscous residue was dissolved in 2 ml MeOH and filtered through a Millipore filter unit. 20 μl of the sample was injected to HPLC.

Extraction of polysaccharides Anthrone-sulfuric acid method was used to extract polysaccharides. 1 g of powdered sample was refluxed three times with 25 ml water for 1 hour. The water extract was filtered while it was hot, and evaporated to about 2 ml in vacuo. 95% EtOH was added to the concentrated extract so that the EtOH concentration was about 85%. The solution was left airtight for 24 hour, and then filtrated with filtering flask. The filtrate cake was washed with 70% EtOH five times and dissolved in water at 60°C. The suspension was centrifuged at 2,000 rpm for 5 min, and the supernatant was made to 100 ml for analysis.

Quantitative analysis The standards used, 7,3'-dihydroxy-4'-methoxyisoflavone 7-*O*-β-D-glucoside; formononetin 7-*O*-β-D-glucoside; (6α*R*, 11α*R*)-3-hydroxy-9,10-dimethoxypterocarpan 3-*O*-β-D-glucoside; 7, 2'-dihydroxy-3', 4'-dimethoxyisoflavan 7-*O*-β-D- glucoside; 7,3'-dihydroxy-4'-methoxyisoflavone (corresponding to isoflavonoids I-V respectively) and astragalosides I-IV, were gifts from Dr. Masaki Anetai of Hokkaido Institute of Public Health in Japan. Dextran of molecular weight 15,000~20,000 (Sigma; St. Louis, MO) was used as a standard for quantitative analysis of

total polysaccharide. Analytical and HPLC grade reagents were used for HPLC.

For the calibration of isoflavonoids I-V and astragalosides I-IV, three injections were performed for each measurment. The standard curve was calibrated by using the linear least- squares regression equation derived from the peak areas. The HPLC was performed on a NOVA-PAK C_{18} column (particle size 4 μm), 3.9 mm × 300 mm in a Waters PC 800 Integrator, Waters 486 Tunable Absorbance Detector and Waters^{TM} 600 Pump. The mobile phases were CH_3CN/H_2O (43:57) for astragalosides and $CH_3CN/H_2O/CH_3COOH$ (270:730:1) for isoflavonoids with a flow rate 1.0 ml/minute at 40°C and detection at 205 or 280 nm.

For polysaccharide calibration, Dextran standard solution was prepared. Then, 4.0 ml 0.2% anthrone-sulfuric acid (prepared just before use) was added. Absorbance at 625 nm was measured after 30 min.

RESULTS AND DISCUSSION

As shown in Table 1, HPLC calibration curves of isoflavonoids, astragalosides and polysaccharide exhibited good linerarity in a range from ~1 μg/ml to ~90 μg/ml. The RSD (relative square difference) was within a range from 2 to 3%. Recovery test by extracting a known amounts of isoflavonoid, astragaloside and polysaccharide showed that the recoveries of the tested isoflavonoids, astragalosides and polysaccharide were from 92 % to 100 %. Fig. 1 A shows typical chromatograms of MeOH extracts of various Astragali Radices. Each of the peaks for isoflavonoids I-V was distinct. Fig. 1B shows similar results on the butanol extracts. The peaks were identified by comparing the retention times and by spiking the sample with stock standard solutions (data not shown). The amounts of isoflavonoids I-V, astragalosides I-IV and total polysaccharide determined in 46 different sources of Huangqi and its adulterants are summarized in Table 2. There was no obvious difference in the concentration of the total isoflavonoid among various species of *Astragalus*. In general, the concentrations of astragalosides I-IV, especially of astragaloside IV in *A. membranaceus var. mongholicus* were higher than those in *A. membranaceus*. *H. polybotrys* contained no detectable amount of astragalosides; however, it contained a high concentration of isoflavonoids II and V. Astragaloside has α and β methyl epimers at 20-carbon[4] and a quantity of β epimer is known to be presented in our test *Astragalus*. However, other

Table 1. Calibration of isoflavonoids, astragalosides and polysaccharide by HPLC

Standard compounds	Regression equation	r	Recovery(%)
Isoflavonoid I	Y=10150X–4718	0.9995	103.38
Isoflavonoid II	Y=15994X–4834	0.9990	97.48
Isoflavonoid III	Y=12357X+1667	0.9999	92.25
Isoflavonoid IV	Y=32496X+41760	0.9997	98.57
Isoflavonoid V	Y=99354X–2743	0.9999	98.28
Astragaloside I	Y=66843X+2332	0.9999	100.54
Astragaloside II	Y=625519X+34960	0.9998	98.61
Astragaloside III	Y=20893X–9114	0.9999	97.28
Astragaloside IV	Y=30590X+6476	0.9999	96.12
Dextran	$Y=7.07\times10^{-3}X+6.72\times10^{-3}$	0.9999	97.48

The range of isoflavonoids, astragalosides and polysaccharide used for standard curves calibration was from ~1 μg/ml to ~90 μg/ml. HPLC performance was described in Materials and Methods. The RSDs were about 2 to 3%, where N=5. Recovery was determined by adding known amount of constituents into the plant, where the amount of active constituents was known. These samples were subjected to HPLC analysis. The molecular weight of Dextran is 18,100. The regression equation was used to calibrate the concentration of various constituents as listed in Table 2.

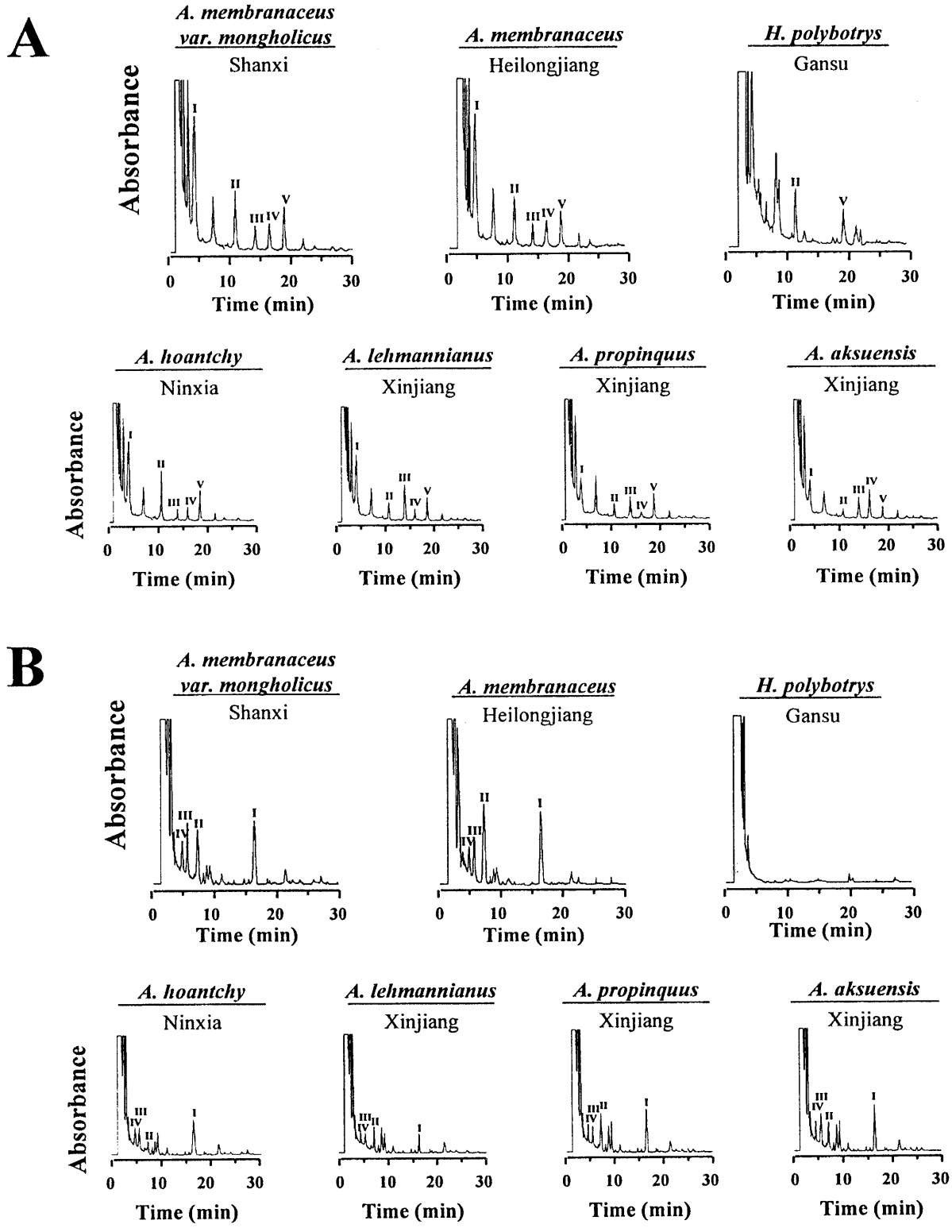

Figure 1. HPLC chromatograms of extracts from Astragali Radixes

Waters PC 800 Integrator, Waters 486 Tunable Absorbance Detector, Waters 600 Pump and NOVA-PAK C_{18} (particle size 4 μm, 3.9 mm i.d. × 300 mm) were used in the HPLC performance. The mobile phase was CH_3CN/H_2O/ CH_3COOH (270:730:1) with a flow rate 1.0 ml/min at 40°C. **(A):** chromatograms of methoanl extract for the determination of isoflavonoids. The indicated peaks from I-V correspond to isoflavonoids I-V. The absorbance was at 280 nm. **(B):** chromatograms of butanol extract for the determination of astragalosides. The mobile phase was CH_3CN/H_2O (43:57). The indicated peaks from I-IV correspond to astragalosides I-IV. The absorbance was at 205 nm. Arbitrary units of absorbance are used. The sources of Astragali Radixes are shown.

Table 2. Active Constituents in Astragali Radixes (Huangqi)

No.	Name	Source	Part	Age (year)	Isoflavonnoids (mg/100g)						Astragalosides (mg/100g)					TP$^{\Delta}$ (%)
					I	II	III	IV	V	T	I	II	III	IV	T	
1	A.membranaceus var. mongholicus	Beijing	root	>3	24	12	8	5	46	95	84	39	9	8	140	7.54
2	ditto	Hebei	root	3	30	11	13	22	42	118	95	42	20	12	169	6.23
3	ditto	Hebei	root	2	41	15	27	13	48	144	88	36	28	17	169	9.60
4	ditto	Hebei	aerial	2	–	–	–	8	6	14	18	9	5	3	35	0.01
5	ditto	Hebei	aerial	3	–	–	8	11	7	26	10	7	4	2	23	0.05
6	ditto	Shanxi	root	3	64	18	30	9	35	156	125	49	11	11	196	5.86
7	ditto	Shanxi	aerial	3	5	–	–	8	6	19	11	4	5	2	22	0.06
8	ditto	Shanxi	root	2 **	82	19	48	19	17	185	146	47	12	22	227	7.28
9	ditto	Shanxi	root	>3*	60	14	21	9	34	138	55	15	7	15	92	13.48
10	ditto	Shanxi	root	2	93	28	10	5	15	151	123	45	10	34	212	7.90
11	ditto	Shanxi	aerial	2	4	–	–	10	8	22	13	6	9	3	31	0.03
12	ditto	Neimenggu	root	3	78	22	39	56	18	213	24	8	4	17	53	8.73
13	ditto	Neimenggu	aerial	3	–	–	6	7	9	22	5	10	4	2	21	0.06
14	ditto	Neimenggu	root	2	39	18	8	13	35	113	96	63	7	25	191	6.05
15	ditto	Neimenggu	aerial	2	6	–	–	8	5	19	6	7	12	4	29	0.01
16	ditto	Ninxia	root	2	23	30	12	8	24	97	75	24	13	7	119	5.10
17	ditto	Heilongjiang	root	1	126	98	7	12	82	325	21	8	4	19	52	4.37
18	ditto	Heilongjiang	aerial	1	4	2	–	9	11	26	10	7	2	4	23	0.03
19	ditto	Heilongjiang	root	2	90	25	45	29	57	246	21	12	5	8	46	4.06
20	ditto	Heilongjiang	aerial	2	–	–	4	10	8	22	8	13	8	4	33	0.02
21	A. membranaceus	Hebei	root	3	26	13	9	27	63	138	84	34	13	10	141	5.26
22	ditto	Hebei	aerial	3	–	3	6	4	12	25	15	10	4	3	32	0.05
23	ditto	Shanxi	root	3	21	4	11	21	43	100	102	39	17	9	167	3.65
24	ditto	Shanxi	aerial	3	–	–	–	12	6	18	13	9	4	2	28	0.07
25	ditto	Shanxi	root	2 **	52	9	25	12	34	132	98	27	7	16	148	3.18
26	ditto	Shanxi	root	2	69	18	29	14	22	152	120	34	10	14	178	8.47
27	ditto	Shanxi	root	>3	77	20	14	8	25	144	88	69	8	9	174	6.35
28	ditto	Shanxi	aerial	2	–	3	–	7	9	19	13	8	6	3	30	0.02
29	ditto	Shanxi	aerial	>3	2	–	–	5	8	15	7	12	2	2	23	0.05
30	ditto	Neimenggu	root	2	60	14	31	9	34	148	125	45	14	11	195	5.77
31	ditto	Neimenggu	aerial	2	–	7	–	10	5	22	5	9	12	3	29	0.01
32	ditto	Neimenggu	root	1	117	44	15	6	20	202	104	84	66	18	272	6.64
33	ditto	Neimenggu	root	3	62	12	9	96	28	179	20	8	25	9	62	7.07
34	ditto	Neimenggu	aerial	3	4	2	–	3	12	21	11	6	2	2	21	0.04
35	ditto	Heilongjiang	root	1	121	38	8	5	19	191	55	13	7	16	91	7.66
36	ditto	Heilongjiang	aerial	1	3	–	–	6	4	13	6	11	4	4	25	0.03
37	ditto	Heilongjiang	root	2	51	14	11	9	43	128	102	39	7	13	161	6.83
38	ditto	Heilongjiang	aerial	2	–	–	–	7	6	13	8	14	9	3	34	0.01
39	ditto	Sichuan	root	>3	34	9	15	21	9	88	86	10	14	7	117	7.19
40	H. polybotrys	Gansu	root	>3*	–	128	–	–	87	215	–	–	–	–	–	14.46
41	ditto	Ninxia	root	>3*	–	95	–	–	22	117	–	–	–	–	–	12.87
42	A. hoantchy	Ninxia	root	>3*	79	62	8	12	35	196	72	4	11	9	96	10.24
43	ditto	Ninxia	aerial	>3*	–	8	–	–	7	15	8	–	3	–	11	0.05
44	A. lehmannianus	Xinjiang	Root	>3*	60	14	43	8	19	144	38	24	8	7	77	9.77
45	A. propinquus	Xinjiang	Root	>3*	23	7	10	5	15	60	93	45	10	11	159	6.87
46	A. aksuensis	Xinjiang	Root	>3*	17	4	16	21	8	39	124	23	36	15	198	8.24

* From natural resources.
** Cultivated area was changed during growth.
$^{\Delta}$TP: total polysaccharide.

astragaloside epimers are not known to be biologically active and in the present assay, only α-epimers were assayed.

Astragalus membranaceus var. mongholicus from Shanxi (sample #10) contained the highest concentration of astragaloside IV, and that from Neimenggu (sample

#32) had the highest level of astragaloside IV of all the tested *A. membranaceus*. Higher concentrations of isoflavonoids I and II were observed in plants younger than 2 years. The plants ≥3 years of age had a higher concentration of polysaccharide than the younger plants. Of the samples tested, wild plants contained a slightly larger amount of active constituents than the cultivated plants (Table 2). Besides, the active constituents were mostly distributed in the root, and scarcely in the aerial part. Regarding polysaccharide, *H. polybotrys* from Gansu (sample #40) contained the highest concentration of polysaccharide. Of the 46 samples tested, the concentrations of isoflavonoids I-V, astragalosides I-IV and total polysaccharide were all higher in *A. membranaceus var. mongholicus* and *A. membranaceus* than in *A. lehmannianus*, *A. hoantchy*, *A. aksuensis* and *A. propinquus*. The latter four species are the common adulterants of Astragali Radix. Both *A. membranaceus var. mongholicus* and *A. membranaceus* could be used as Astragali Radix, and indeed, they have a close resemblance in morphology[5], ribosomal RNA sequence[8] and chemical profiles as demonstrated here.

Astragali Radix is cultivated also in South East Asia, Korea and Japan. Chemical analysis of the amounts of five isoflavonoids, four astragalosides, γ-aminobutyric acid, and molar ratio of L-canavanine to L-arginine in those Astragali Radices from Japan (Hokkaido, Ibaraki), China (Beijing, Nanjing, Shanxi, Neimonggu, Hongkong, Taibei, Shaanxi, Guangxi, Chengdu), D. P. R. Korea and R. Korea are reported by Anetai et al.[7,9]

The level of isoflavonoids and astragalosides in our studies are comparable to their results. However, the materials they used were market samples bearing the name of Huangqi and the species origin was not identified. In the present study, samples were collected by us from different regions of China, and their species origins were identified. Therefore, the best farming areas and conditions to produce Astragali Radix could be determined. In conclusion, Astragali Radix from Shanxi was the one that contained significantly higher amounts of isoflavonoids, saponins and polysaccharide. The present results will be helpful for the quality control of Astragali Radix.

Acknowledgements: We are grateful to Dr. Masaki Anetai of Hokkaido Institute of Public Health in Japan for presenting *Astragalus* isoflavonoids I-V and astragalosides I-IV, and to Prof. C. Y. Yang for field assistance in China. The research was supported by grants from BRI at HKUST (Chinachem Group, ISF AF-178-97 and AF-150-99 to K.W.K.T.) and the Foundation of Chinese Natural Sciences (#39730500 to J. A. D.).

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