

## Chemical Analysis of Radix Astragali (Huangqi) in China: A Comparison with Its Adulterants and Seasonal Variations

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Radix Astragali (root of *Astragalus*; Huangqi) is a popular traditional Chinese medicine, and *Astragalus membranaceus* and *A. membranaceus* var. *mongolicus* are two commonly used species; however, there are many *Astragalus* species that could act as adulterants of Radix Astragali. To find the chemical composition of Radix Astragali, the main constituents including flavonoids, saponins, polysaccharides, amino acids, and trace elements were determined in two Radices Astragali, *A. membranaceus* and *A. membranaceus* var. *mongolicus*, and its eight adulterants, *Astragalus propinquus*, *Astragalus lepsensis*, *Astragalus aksuensis*, *Astragalus hoantchy*, *Astragalus hoantchy* subsp. *dshimensis*, *Astragalus lehmannianus*, *Astragalus sieversianus*, and *Astragalus austrosibiricus*. The results showed that the amounts of main constituents such as isoflavonoids and astragalosides varied in different species. In distinction, *A. membranaceus* and *A. membranaceus* var. *mongolicus* contained a higher amount of astragaloside I and IV. In addition, the main constituents of *A. membranaceus* var. *mongolicus* changed according to seasonal variation and age of the plant. The chemical composition of different species of *Astragalus* would provide useful information for the quality control of Radix Astragali.

**KEYWORDS:** Astragalosides; HPLC; isoflavonoids; traditional Chinese medicine

### INTRODUCTION

Radix Astragali (root of *Astragalus*; Huangqi), a common traditional Chinese medicine, has been proved to be an immunostimulant, tonic (adaptogenic), hepatoprotective, diuretic, antidiabetic, analgesic, expectorant, and sedative drug (1–3). Although Radix Astragali has a long history of medicinal use in Chinese herbal medicine, its pharmacological properties and clinical applications have not been studied until recently. Radix Astragali has been demonstrated to have a wide range of immunopotentiating effects and has been used as an adjunct medicine during cancer therapy (4). Demand for Radix Astragali is enormous throughout the world, particularly in the market of Southeast Asia and Japan. The genus *Astragalus* L. is comprised of 278 species, two subspecies, 35 varieties, and two forma in China (5), of which 12 carry the name Huangqi on the market and are cultivated in more than 10 different regions

in China. Radices of *Astragalus membranaceus* (Fischer) Bunge and *A. membranaceus* (Fisch.) Bunge var. *mongolicus* (Bunge) P. K. Hsiao (6) are authentic botanical sources of Radix Astragali in Chinese Pharmacopoeia (1, 2) and monographs (4). Indeed, *A. membranaceus* and *A. membranaceus* var. *mongolicus* share a great similarity in their main constituents (7) and genomic DNA sequences (8).

The constituents most often associated with the activity of Radix Astragali are isoflavonoids, saponins, polysaccharides,  $\gamma$ -aminobutyric acid (GABA), and various trace elements (3, 4). Astragaloside IV is normally used as a marker for quality control. The levels of these main constituents in Radix Astragali determined by scanning thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and a colorimetric method were shown to vary according to their origin (4, 7, 9, 10). Today, Radix Astragali is mostly prepared from cultivated plants. For example, *A. membranaceus* and *A. membranaceus* var. *mongolicus* are cultivated mainly in the northern part (Shanxi, Neimenggu, and Hebei) and the northeastern part (Heilongjiang) of China. Recent studies also indicate that Shanxi of China produces the best quality of Radix Astragali (7). Although the species origin of Radix Astragali has been defined,

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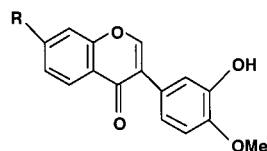
<sup>§</sup> Shanghai Second Medical University.

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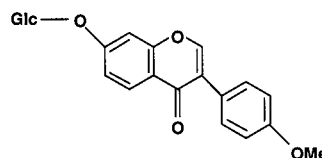
## Isoflavonoids

1. R = O - Glc

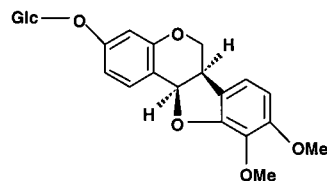
5. R = OH



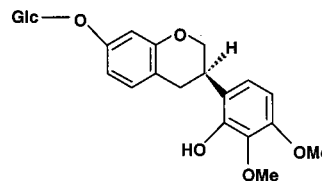
2.



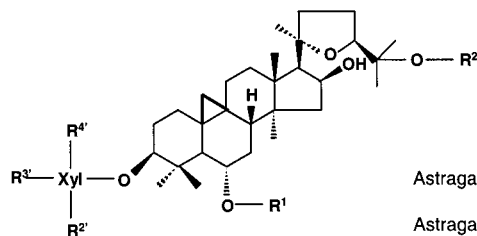
3.



4.



## Astragalosides



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>
Astragaloside I	Glc	H	Ac	Ac	H
Astragaloside II	Glc	H	Ac	H	H
Astragaloside III	H	H	Glc	H	H
Astragaloside IV	Glc	H	Glc	H	H

**Figure 1.** Chemical structures of isoflavonoids and astragalosides. Isoflavonoids 1–5 and astragalosides I–IV are shown here. Side groups of Ac (acetate), Glc (glucose), and H (hydrogen) are indicated.

there are about eight more species of *Astragalus* and a red substitute *Hedysarum polybotrys* Handel-Mazzetti, commonly found in northern China, that could carry the name of Radix Astragali and act as adulterants on the commercial market. The morphological appearances of Radix Astragali and its adulterants show a great resemblance. Therefore, the quality control of Radix Astragali is a serious problem. To compare the quality of Radix Astragali from its adulterants, the levels of isoflavonoids 1–5, astragalosides I–IV, polysaccharides, amino acids, and trace elements in different *Astragalus* species, as well as Radix Astragali collected from different seasons and ages, were determined by reverse phase HPLC and spectrophotometry.

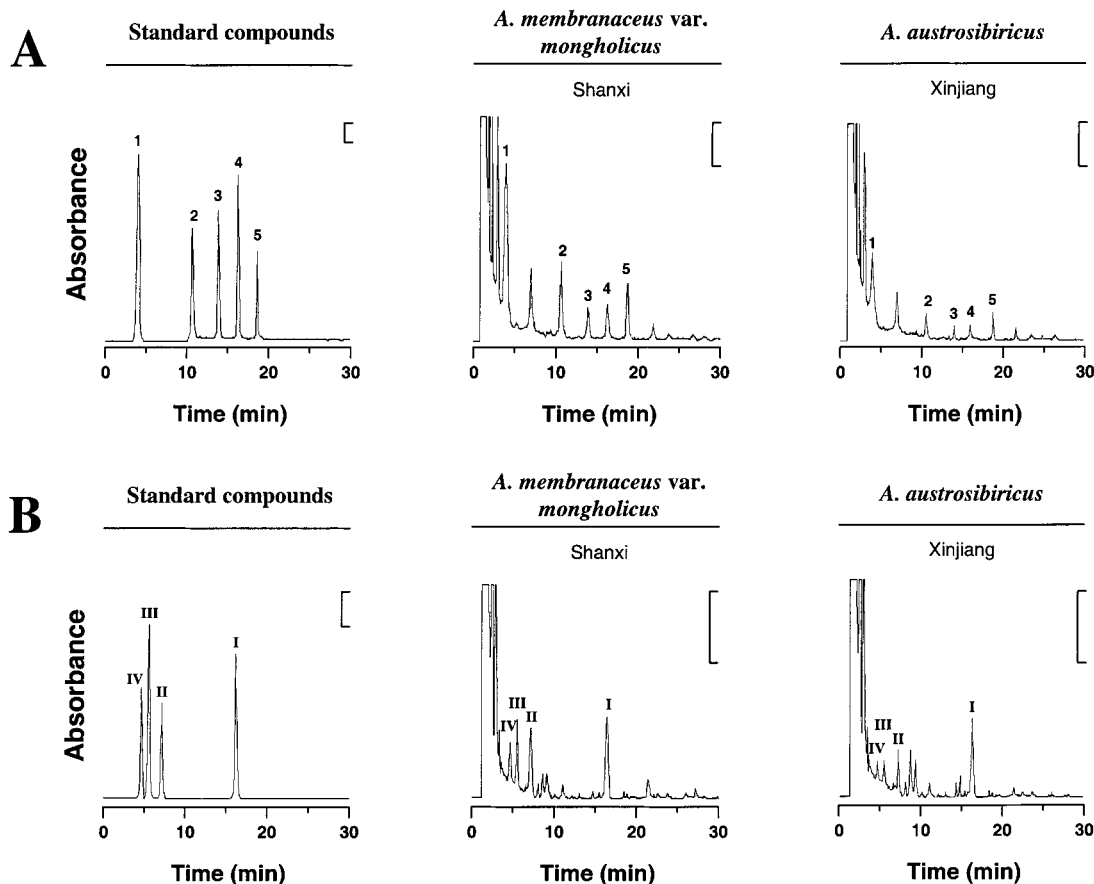
## MATERIAL AND METHODS

**Plant Materials.** All materials were collected from China. Voucher specimens were deposited in the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China. *A. membranaceus* (Fisch.) Bunge (99-01-102) was obtained from Heilongjiang; *A. membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) P. K. Hsiao (99-01-089) was obtained from Neimengu; *Astragalus hoantchy* Franch. (99-04-011) was obtained from Ninxia; *A. hoantchy* Franch. subsp. *dshimensis* (Gontsch.) K. T. Fu (99-04-438), *Astragalus propinquus* Schischk. (99-02-038), *Astragalus aksuensis* Bunge (99-05-255), *Astragalus lepsensis* Bunge (99-06-482), *Astragalus sieversianus* Pall. (99-07-199), *Astragalus lehmannianus* Bunge (99-08-103), and *Astragalus austrosibiricus* Schischk. (99-09-211) were obtained from Xinjiang. *H. polybotrys* Handel-Mazzetti (99-01-039), a red adulterant of Radix Astragali, was from Gansu. Different *Astragalus* species from geographical properties at the same region were

collected and dried under the sun. The chosen area was based on the popularity of individual *Astragalus* that was found in China. About 10 batches of individual species having similar but not identical geographical properties at the same region were tested. Samples of different growth seasons and ages were collected by the same method from the same geographical regions. Individual samples were prepared from ~500 g of powder that was ground from ~20 plants of the same population. This grinding process was done during the field collection before they were delivered to the laboratory. The collected powder was stored with silica gel, which stabilized the chemical constituents.

**Extraction of Chemical Constituents.** For isoflavonoids and saponins, 5 g of ground powder was extracted three times in a Soxhlet with 100 mL of aqueous MeOH (MeOH/H<sub>2</sub>O, 4/1) for 2 h (7). The combined MeOH extract was filtered and evaporated to dryness in vacuo. For the isoflavonoid assay, the viscous residue was stirred in 25 mL of hot water, and the suspension was treated with 10, 7.5, and then 5 mL of ethyl acetate. The aqueous lower phase was discarded. The combined ethyl acetate phases containing isoflavonoids (~12.5 mL) were evaporated to dryness in vacuo. Saponins were analogously extracted by using *n*-butanol saturated with water, and the butanol extract was concentrated. Both viscous residues were dissolved in 2 mL of MeOH and filtered through a Millipore filter unit. Twenty microliters of the sample was injected to HPLC.

The anthrone–sulfuric acid method was used to extract polysaccharides. One gram of ground powder was refluxed three times with 25 mL of water for 1 h. The water extract was filtered while it was hot. The filtrate was evaporated to about 2 mL in a vacuum, and then, 95% ethanol was added to the extract until ethanol was about 85%. The solution was kept airtight for 24 h, and then, it was filtrated under vacuum. The cake was washed with 70% ethanol five times and



**Figure 2.** HPLC chromatograms of extracts from Radices Astragali and its adulterants. (A) Chromatograms of methanol extract for the determination of isoflavonoids. The indicated peaks from one to five correspond to isoflavonoids 1–5, respectively. The scale bar indicates the absorbance of 0.01 at 280 nm. (B) Chromatograms of butanol extract for the determination of astragalosides. The indicated peaks from I to IV correspond to astragalosides I–IV, respectively. The scale bar indicates the absorbance of 0.01 at 205 nm. The sources of Radices Astragali are shown.

dissolved in water at 60 °C. The solution was centrifuged at 2000 rpm for 5 min to remove the insoluble matter. The supernatant was adjusted to 100 mL in a volumetric flask and stored for analysis.

For the extraction of amino acids, 100 mg of ground powder was soaked with 10 mL of 6 N HCl at 110 °C for 24 h, evaporated to dryness, and then diluted 25 times by 0.01 N HCl and stored for analysis. For water soluble extract, 5 g of ground powder was extracted three times with 100 mL of water with 6 h of airtight shaking and then left for 18 h at room temperature. The combined extracts were filtered and evaporated to dryness for weighing.

**Quantitative Analysis of Main Constituents.** Standards including 7,3'-dihydroxy-4'-methoxyisoflavone 7-*O*- $\beta$ -D-glucoside (isoflavonoid 1); formononetin 7-*O*- $\beta$ -D-glucoside (isoflavonoid 2); (6 $\alpha$ R,11 $\alpha$ R)-3-hydroxy-9,10-dimethoxypterocarpan 3-*O*- $\beta$ -D-glucoside (isoflavonoid 3); 7,2'-dihydroxy-3',4'-dimethoxyisoflavan 7-*O*- $\beta$ -D-glucoside (isoflavonoid 4); 7,3'-dihydroxy-4'-methoxyisoflavone (isoflavonoid 5); and astragalosides I–IV (Figure 1) were gifts from Dr. Masaki Anetai of the Hokkaido Institute of Public Health, Japan. Dextran with a molecular weight of 15 000–20 000 (Sigma, St. Louis, MO) was used as a standard for quantitative analysis of *Astragalus* total polysaccharide. AR and HPLC grade reagents were from Sigma.

In the quantitation of isoflavonoids and astragalosides, the standards were weighed and dissolved in 1 mL of methanol to give serial concentrations. Three injections were performed for each dilution. The standard curve was calibrated by using the linear least-squares regression equation derived from the peak area (7). The HPLC was performed on a 300 mm  $\times$  3.9 mm i.d., 4  $\mu$ m Nova Pak C<sub>18</sub> column with Waters PC 800 integrator, Waters 486 tunable absorbance detector, and Waters 600 pump. The mobile phases were CH<sub>3</sub>CN/H<sub>2</sub>O (43:57) for astragalosides or CH<sub>3</sub>CN/H<sub>2</sub>O/CH<sub>3</sub>COOH (270:730:1) for isoflavonoids with a flow rate of 1.0 mL/min at 40 °C and detection at 205 or 280 nm.

For polysaccharide calibration, dextran was weighed and dissolved in 100 mL of water to give serial concentrations. Standard solutions (0.6 mL) or prepared samples were taken and adjusted to a volume of 2.0 mL. Then, 4.0 mL of 0.2% anthrone–sulfuric acid (prepared just before use) was added. Absorbance at 625 nm was measured after 30 min of color reaction. For amino acid determination, a Hitachi 835-50 auto amino acid detector, 150 mm  $\times$  2.6 mm ion-exchange column at 53 °C and 10 297 kPa, flow rate 0.225 mL/h, running time 70 min, and 50  $\mu$ L sample, was used. Seventeen amino acids were determined as follows: GABA; arginine (Arg); proline (Pro); lysine (Lys); histidine (His); aspartic acid (Asp); threonine (Thr); serine (Ser); glutamic acid (Glu); tyrosine (Tyr); phenylalanine (Phe); isoleucine (Ile); leucine (Leu); glycine (Gly); alanine (Ala); valine (Val); and methionine (Met). Others were not determined. To extract trace elements, a Mark 1100 series plasma laser (Jarrell-Ash Com., U.S.A.) was used. Two grams of powder was put in a white crucible with the temperature maintained at 700 °C for incineration and then decomposed by hydrochloric acid, nitric acid, and hydrofluoric acid. The prepared solution was applied in plasma torch for detection.

## RESULTS AND DISCUSSION

The HPLC calibration curves of isoflavonoids, astragalosides, and polysaccharides exhibited good linearity in a range from  $\sim$ 1 to  $\sim$ 90  $\mu$ g/mL, which had been calibrated previously (7). The RSD was within a range from 2 to 3%. A recovery test by extracting known amounts of isoflavonoid, astragaloside, and polysaccharides showed that the recoveries of the tested compounds were from 92 to 100%. Figure 2A shows typical chromatograms of MeOH extracts of roots from *A. membranaceus* var. *mongolicus* and *A. austrosibiricus*. The peaks for

Table 1. Main Constituents in Radices Astragali (Huangqi)<sup>a</sup>

species source	isoflavonoids (mg/100 g)					TIS (%)	astragalosides (mg/100 g)				TAS (%)	TP (%)	TAA (%)	WSA (%)
	1	2	3	4	5		I	II	III	IV				
<i>A. membranaceus</i> Heilongjiang*	51	14	11	9	43	0.75	102	39	7	13	1.96	6.83	0.44	23.7
<i>A. membranaceus</i> var. <i>mongholicus</i> Shanxi*	93	28	10	5	15	1.12	123	45	10	34	2.64	7.90	0.36	29.6
<i>A. membranaceus</i> var. <i>mongholicus</i> Shanxi	97	32	15	8	31	1.49	130	48	11	39	3.58	13.48	0.60	41.6
<i>A. propinquus</i> Xinjiang	23	7	10	5	15	0.98	93	45	10	11	2.23	6.87	0.35	36.4
<i>A. lepsensis</i> Xinjiang	20	11	24	7	26	0.84	75	38	24	7	2.02	7.15	0.28	44.5
<i>A. aksuensis</i> Xinjiang	17	4	16	21	8	1.62	124	23	36	15	2.78	8.24	0.64	32.8
<i>A. hoantchy</i> Ninxia	79	62	8	12	35	0.97	72	4	11	9	1.66	10.24	0.52	38.5
<i>A. hoantchy</i> subsp. <i>dshimensis</i> Xinjiang	56	49	10	7	28	0.84	68	5	24	6	1.59	12.2	0.57	43.7
<i>A. lehmannianus</i> Xinjiang	60	14	43	8	19	1.81	38	24	8	7	2.16	9.77	0.33	40.6
<i>A. sieversianus</i> Xinjiang	34	8	9	11	22	1.56	49	36	13	8	2.58	7.43	0.58	32.5
<i>A. austrosibiricus</i> Xinjiang	18	12	7	5	13	1.25	86	28	8	4	1.35	5.68	0.46	29.6
<i>Hedysarum polybotrys</i> Sichuan		128			87	1.29						14.46	0.36	30.5
<i>H. polybotrys</i> Ningxia		95			22	0.94						12.87	0.28	28.9

<sup>a</sup> Samples were collected from the end of August to early September. \*, from cultivated sources. TIS, total isoflavonoids in percent of total dry weight; TAS, total astragalosides in percent of total dry weight; TP, total polysaccharides in percent of total dry weight; TAA, total amino acids in percent of total dry weight; WSA, water soluble extract in percent of total dry weight. Values are means,  $n = 10$ .

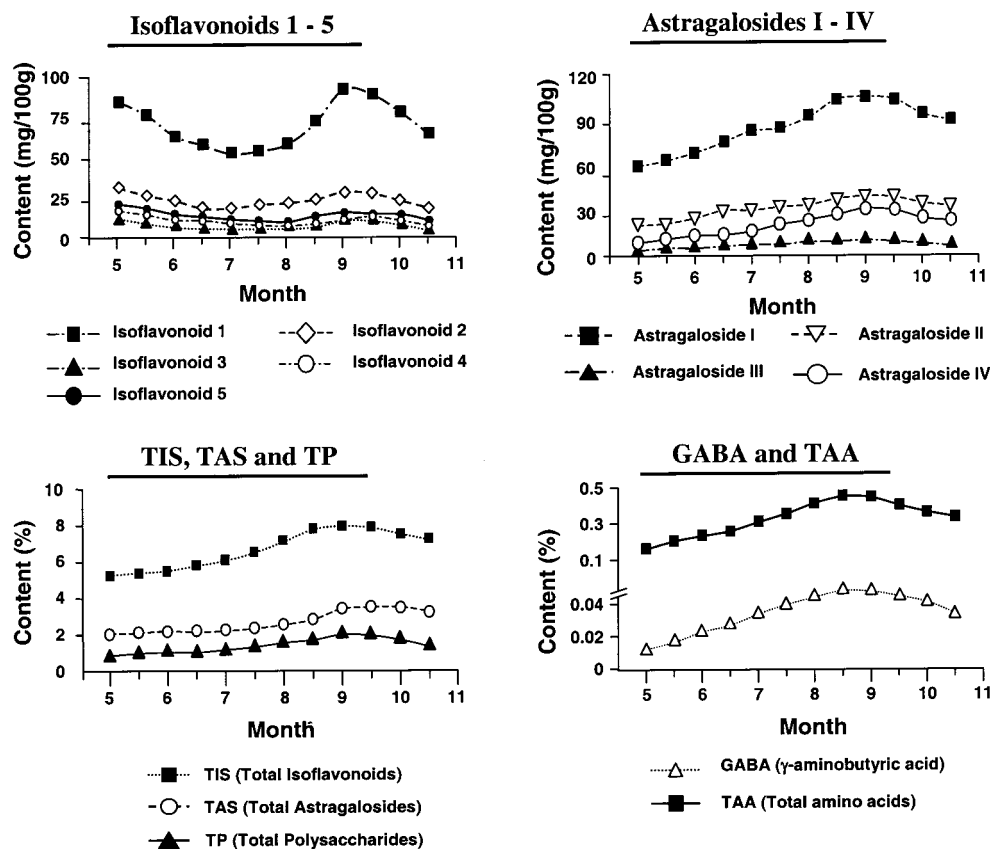
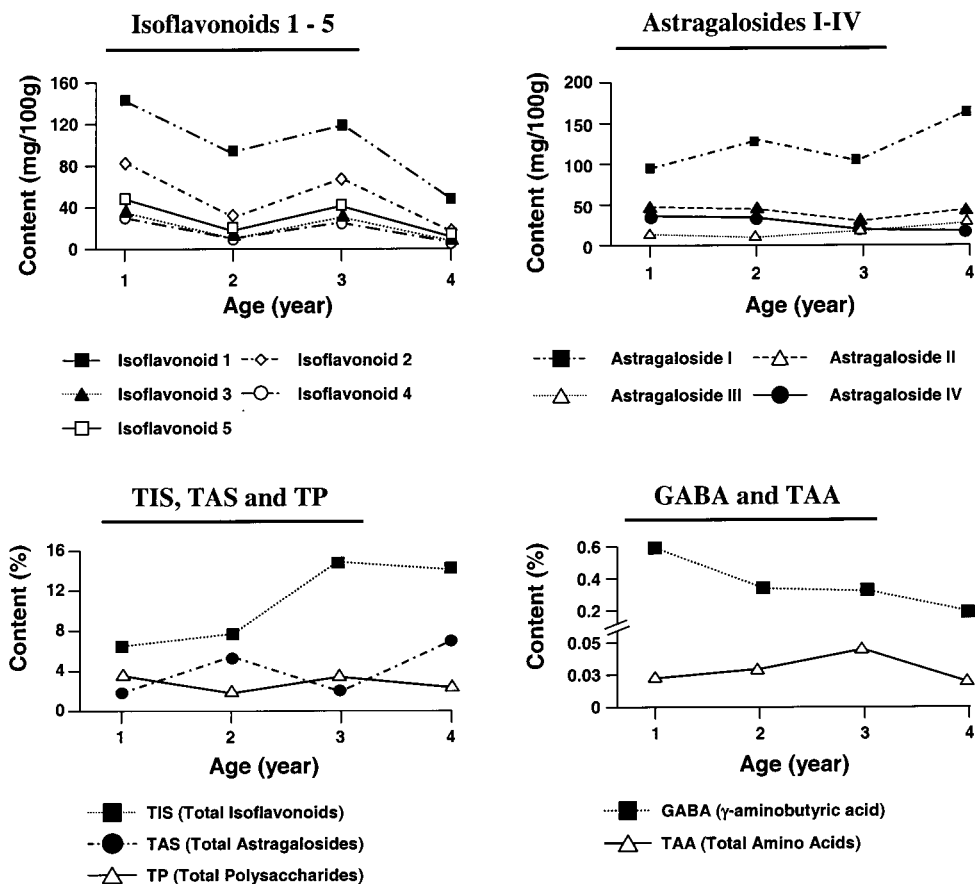


Figure 3. Seasonal change of chemical composition in Radix Astragali. Cultivated *A. membranaceus* var. *mongholicus* from the Shanxi region was used for the analysis, and they were 2–3 years old, depending on time of harvest. Values are means, where SEM is not shown for clarity, which is  $\leq 10\%$  of the means,  $n = 5$ .



**Figure 4.** Effect of age on the chemical composition of *Radix Astragali*. *A. membranaceus* var. *mongholicus* from cultivated sources in the Shanxi region was used for the analysis, harvested in the fall. Values are means, where SEM is not shown for clarity, which is  $\leq 10\%$  of the means,  $n = 5$ .

isoflavonoids were distinct. **Figure 2B** shows similar results on the butanol extracts showing the peaks of astragalosides. Peaks were identified by two means: (i) by comparing the retention times of the unknown peaks with those of the standards eluted under the same conditions and (ii) by spiking the sample with stock standard solutions of isoflavonoids or astragalosides (data not shown). The amounts of isoflavonoids, astragalosides, total polysaccharides, and total amino acids were determined in *A. membranaceus* and *A. membranaceus* var. *mongholicus* and its eight adulterants: *A. propinquus*, *A. lepsensis*, *A. aksuensis*, *A. hoantchy*, *A. hoantchy* subsp. *dshimensis*, *A. lehmannianus*, *A. sieversianus*, *A. austrosibiricus*, and a red adulterant *H. polybotrys* (**Table 1**). These *Astragalus* species are commonly found in northern China. There was no obvious difference in the concentrations of total isoflavonoids, polysaccharides, and amino acids among different species of *Astragalus*. However, the concentrations of astragalosides, especially of astragaloside I and IV, in *A. membranaceus* and *A. membranaceus* var. *mongholicus* were significantly higher than in other *Astragalus* species, the difference attaining 3–4-folds. Roots of *H. polybotrys* showed a much lower content of isoflavonoids and astragalosides. *A. membranaceus* var. *mongholicus* from a natural source in Shanxi contained a slightly higher content of isoflavonoids and astragalosides than the one from cultivated sources.

In the determination of amino acids and trace elements compositions, all of the tested *Astragalus* species showed similar characteristics (see Supporting Information). Both *A. membranaceus* and *A. membranaceus* var. *mongholicus* did not show any significant higher content of amino acids or trace elements, which suggested that both amino acids and trace elements should

not be considered as chemical markers for the distinction of *Radix Astragali*.

The levels of the main constituents in *Radix Astragali* were changed according to the month of sample collection and the age of the plant (**Figure 3**). September to October was the best period to collect the plants, because they contained the highest concentration of the main constituents. Among these constituents, isoflavonoid 1, astragaloside I, and GABA were rather distinct; the highest concentration of these compounds was found in September in China. Indeed, fall is the optimum time for formation of reproductive organs such as flowers and fruits in *Astragalus*, which may, therefore, accumulate the constituents in the plant. In addition, *Astragalus* is a perennial plant, and the levels of constituents change according to age (**Figure 4**). For instance, total polysaccharides and GABA were significantly higher in the 3-year-old plant, while the 1-year-old plant contained the highest amount of total amino acids. Regarding the cost and the quality, a 3-year-old *Astragalus* seemed to be the best age for harvesting, which showed higher contents of isoflavonoid 1, astragaloside I, GABA, and total polysaccharides.

Precise identification of crude herb is a prerequisite for chemical and pharmacological investigations of traditional Chinese medicine. *A. membranaceus* and *A. membranaceus* var. *mongholicus* are the recognized species of *Radix Astragali* (2), while other *Astragalus* species could act as adulterants. Indeed, *A. lehmannianus* and *A. hoantchy* are common examples of adulterants on the market. As indicated by our results, the contents of main constituents including isoflavonoids, astragalosides, amino acids, and trace elements were rather similar except that astragaloside I and IV were higher in *A. membrana-*

*ceus* and *A. membranaceus* var. *mongholicus*. Therefore, the chemical composition of Radix Astragali could not be used as a conclusive distinction. Instead, studies from our laboratory show that authentic identification by their genomic DNA sequences was more indicative (8).

Besides China, Radix Astragali is cultivated also in Southeast Asia and Japan. The main constituents in those Radices Astragali from different habitat regions of Japan and Southeast Asia have been determined (7, 9, 10). In Japanese Pharmacopoeia (11), substitutes including *Astragalus chrysopterus* Bunge, *Astragalus floridus* Benth, and *Astragalus tongolensis* Ulbr are officially permitted but are not accepted in China. Among these habitats, Radix Astragali from Shanxi, China, and Hokkaido, Japan, contained the highest concentration of astragalosides, and indeed, they are the most expensive herbs on the market. Our results further suggest that 3-year-old *Astragalus* harvested in the fall should be the best quality. However, our studies analyzed only *A. membranaceus* var. *mongholicus* collected from one geographical region, i.e., Shanxi. Therefore, Radix Astragali from other regions of China may show different variations of chemical constituents according to season and age. Nevertheless, Shanxi of China is the largest producer of Radix Astragali on the present commercial market.

The pharmacological effects of *Astragalus* are believed to be derived from flavonoids and saponins. Polysaccharides are also one of the key ingredients, which has been shown to stimulate the immune response (1–3). In addition, a glycan named as AMem-P was isolated from *A. membranaceus*, which showed a strong reticuloendothelial system-potentiating activity in the carbon test (12). Although the total polysaccharides were analyzed in our and other studies (9, 10), the identity of different types of polysaccharides within *Astragalus* remained unclear.

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**Supporting Information Available:** Tables of amino acid composition and trace element contents. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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