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Quality evaluation of Radix Astragali from different sources in China

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Abstract: Radix Astragali (Huangqi) is one of the most valuable traditional Chinese medicinal herbs. It is used in the traditional Chinese medicine to reinforce 'qi', and it has immunostimulant, tonic, and antioxidant activities. There are many different sources of Huangqi in the market. In this study, the quality of Huangqi was evaluated by the measurement of four flavonoids, calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin and formononetin with HPLC-UV, as well as astragaloside IV with HPLC-ELSD. Samples included different plant species, different places of cultivation, different ages of plants, different seasons of collection and different commercial specifications. The results showed that the contents of isoflavonoids and astragaloside IV varied significantly in different sources. Our study provided useful information for the quality evaluation of Radix Astragali.
Keywords: Radix Astragali; Quality evaluation; High performance liquid chromatography; Isoflavonoids; Astragaloside IV

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1. Introduction

Radix Astragali (RA), known as Huangqi in China, is a commonly used traditional Chinese medicinal herb. It has been demonstrated to have immunostimulant, tonic, antioxidant, hepatoproective, diuretic, antidiabetic, anticancer, expectorant, analgesic and sedative activities[1–4]. RA is prepared from the dry roots of *Astragalus membranaceus* and *A. membranaceus* var. *mongholicus*[5]. It contains a number of chemical constituents associated with its pharmacological activities, such as isoflavonoids, saponins, polysaccharides, γ-aminobutyric acid (GABA), and various trace elements[6]. Astragaloside IV is normally used as a chemical marker for quality control. Pharmacology studies have shown that the isoflavonoids and saponins in RA are responsible for its therapeutic effects[7–14]. Therefore, four major isoflavonoids (calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin) together with a saponin (astragaloside IV), were used as marker compounds for evaluating the quality of RA in our study[15].

Today, RA is mostly obtained from cultivated plants, as wild plants are increasingly scarce. *A. membranaceus* is mainly cultivated in Heilongjiang and Sichuan of China. And *A. membranaceus* var. *mongholicus* is cultivated mainly in the Northern Provinces (Shanxi, Neimenggu, Hebei and Gansu). In recent years, most of the herb available in the market is the roots of *A. membranaceus* var. *mongholicus*; however, their place of cultivation, age of plant at harvest, and season of collection are different, and these factors might affect the quality of RA. There are commercial specifications of RA in the herb market in China. The herb suppliers usually divide the herb into one-class specification, two-class specification and three-class specification by the diameter of the root of RA. Plants in one-class specification have the thickest diameter. But there is no report on the scientific basis of these commercial specifications. To provide a more reasonable index and protocol for the quality evaluation of RA, a method for quantitative comparison based on analysis of chemical constituents is desirable. In the present study, RA samples obtained from different sources were collected, and the contents of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin and astragaloside IV were determined by HPLC for quality evaluation.

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2. Experimental

2.1. Chemicals and materials

2.1.1. Chemicals

HPLC grade acetonitrile (CH_3CN) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purified by Milli-Q system (Bedford, MA). Phosphoric acid (H_3PO_4) for analysis was of analytic grade from Beijing Reagent Company (Beijing, China).

2.1.2. Plant materials

All plant materials of RA were purchased from major cultivation centers in 8 Provinces in China, the details are listed in Table 1. The plant materials were identified by Dr. Hu-biao Chen, School of Chinese Medicine, Hong Kong Baptist University. The voucher specimens are deposited at the Herbarium, School of Pharmaceutical Sciences, Peking University Health Science Center.

Table 1. List of plant materials

Species source: *A. membranaceus* var. *mongholicus*			
No.	Date of collection	Location	Notes
1	2005.07	Hunyuan, Shanxi	A-2-a
2	2005.07	Hunyuan, Shanxi	A-3-s
3	2006.01	Chayouhouqi, Neimenggu	A-2-s
4	2006.01	Chayouhouqi, Neimenggu	A-2-a
5	2005.08	Datong, Qinghai	A-2-a
6	2005.08	Datong, Qinghai	A-2-s
7	2005.08	Wuchuan, Neimenggu	A-1-a
8	2005.08	Wuchuan, Neimenggu	A-2-a
9	2005.08	Chayouhouqi, Neimenggu	A-1-a
10	2005.08	Zhangxian, Gansu	A-2-a
11	2005.08	Zhangxian, Gansu	A-1-a
12	2005.08	Guyang, Neimenggu	A-1-a-first-class
13	2005.08	Guyang, Neimenggu	A-1-a-second-class
14	2005.08	Guyang, Neimenggu	A-1-a-third-class
15	2005.08	Guyang, Neimenggu	A-2-a-first-class
16	2005.08	Guyang, Neimenggu	A-2-a-second-class
17	2005.08	Guyang, Neimenggu	A-2-a-third-class
18	2005.07	Hunyuan, Shanxi	A-2-a-first-class
19	2005.07	Hunyuan, Shanxi	A-2-a-second-class
20	2005.07	Hunyuan, Shanxi	A-2-a-third-class
21	2005.08	Chifeng, Neimenggu	A-2-a
22	2005.08	Keshiketengqi, Neimenggu	C-a
23	2005.08	Wuchuan, Neimenggu	A-2-a
24	2005.08	Wuchuan, Neimenggu	A-2-a
25	2005.08	Guyang, Neimenggu	A-2-a
26	2005.08	Guyang, Neimenggu	A-3-a
27	2005.08	Hangjinqi, Neimenggu	C-a
28	2005.08	Dingxi, Gansu	A-2-a
29	2005.08	Longxi, Gansu	A-2-a
30	2005.08	Longxi, Gansu	A-2-a
31	2005.08	Longxi, Gansu	A-2-a
32	2005.08	Longxi, Gansu	A-2-a
33	2005.08	Weiyuan, Gansu	A-2-a
34	2005.08	Tanchang, Gansu	A-2-a
35	2005.08	Tanchang, Gansu	A-2-a
36	2005.08	Minxian, Gansu	A-2-a
37	2005.08	Minxian, Gansu	A-2-a
38	2005.08	Minxian, Gansu	A-2-a
39	2005.08	Shandan, Gansu	A-2-a
40	2005.08	Gangu, Gansu	A-3-a
41	2005.08	Lintao, Gansu	A-2-a
42	2005.08	Datong, Qinghai	A-2-a
43	2005.08	Ping'an, Qinghai	A-2-a
44	2005.08	Guide, Qinghai	A-2-a
Species source: *A. membranaceus*			
No.	Date of collection	Location	Notes
45	2005.09	Tangyuan, Heilongjiang	C-a
46	2005.09	Tangyuan, Heilongjiang	C-a
47	2005.09	Jiamusi, Heilongjiang	C-a
48	2005.09	Tahe, Heilongjiang	C-a
49	2005.09	Tahe, Heilongjiang	C-a
50	2005.08	Elunchunqi, Neimenggu	C-a
51	2005.08	Elunchunqi, Neimenggu	C-a
52	2005.09	Luobei, Heilongjiang	C-a
53	2005.09	Wendeng, Shandong	A-3-a
54	2005.08	Elunchunqi, Neimenggu	A-8-a
55	2005.07	Xunyi, Shanxi	A-2-a
56	2005.09	Anguo, Hebei	A-1-a

Notes include A: cultivated, B: wild, C: semi-wild; 1–8: ages of plants; a: autumn collection; s: spring collection.

2.1.3. Reference standards

Standards including calycosin-7-*O*-β-D-glucopyranoside (1), ononin (2), calycosin (3), formononetin (4) and astragaloside IV (5) (Fig. 1) were separated from *A. membranaceus* and characterized by chromatography and spectral methods. The purity of the five standards was greater than 98% by the peak normalization method using HPLC-UV or HPLC-ELSD.

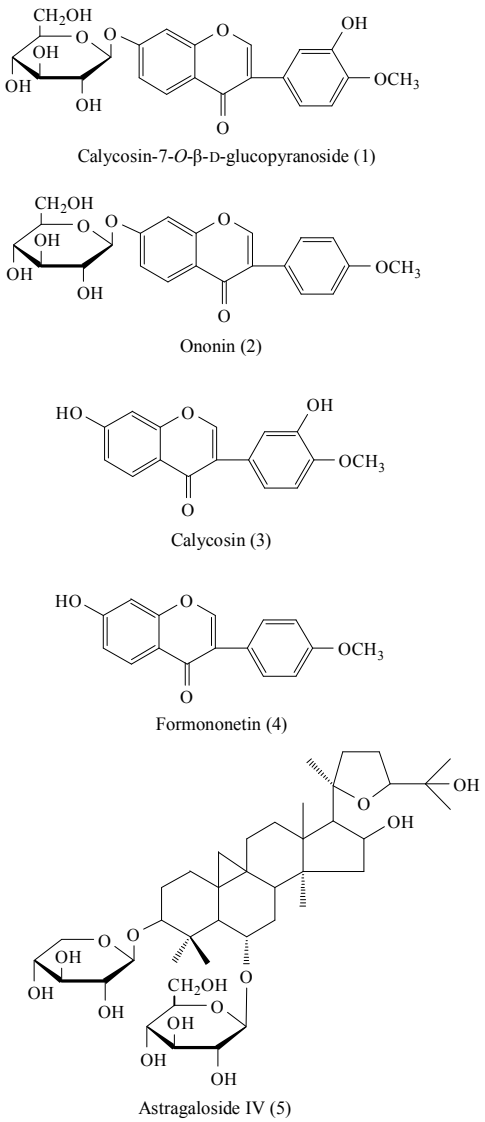

Figure 1. Structures of compounds 1–5.

2.2. Apparatus

A Jasco HPLC system (Tokyo, Japan), equipped with a quaternary pump, a diode array spectrophotometric dectector (DAD), and a column oven, was used for the analysis of the flavonoids. HPLC/ELSD

analysis for astragaloside IV was performed on a Shimadzu LC-10AT vp liquid chromatograph system (Shimadzu Co., Japan) consisting of a quaternary pump, a column oven, and an ELSD-2000 detector (Alltech, USA) couple with CLASS-VP workstation. The column was a YMC-Pack ODS (250 mm×4.6 mm, 4 μm) analytical column (YMC, Japan). An ultrasonic cleaner KQ-500DB (KunShan, China) was used for extraction.

2.3. Quantitative analysis of main constituents

2.3.1. Preparation of sample solutions

Representative samples were cut into smaller pieces, further ground into power, and passed through a 40-mesh sieve. For isoflavonoid assay, 2 g of the ground powder was extracted two times in an ultrasonic processor in 50 mL aqueous MeOH (MeOH–H_2O, 1:1) for 45 min[15,16]. The combined MeOH extract was filtered and evaporated to dryness in vacuo. The viscous residue was stirred in 20 mL water and treated with 20 mL *n*-butanol twice. The combined *n*-butanol extract was concentrated. The viscous residue was dissolved in 5 mL aqueous MeOH (MeOH–H_2O, 1:1) and filtered through a Millipore filter. Twenty microliters of the sample was injected to HPLC.

For astragaloside IV assay, 2 g of the ground powder was extracted with 90 mL aqueous MeOH (MeOH–H_2O, 1:1) under reflux for 4 h. The filtered extract was evaporated to dryness in vacuo[15]. The viscous residue was stirred in 20 mL water and treated with 20 mL *n*-butanol twice. The combined *n*-butanol extract was concentrated. The viscous residue was dissolved in 5 mL water, and then loaded onto a HP-20 column followed by washing with 30 mL water to remove polysaccharides. The column was eluted with 100 mL aqueous EtOH (EtOH–H_2O, 95:5). The EtOH eluate was collected and concentrated. The viscous residue was dissolved in 5 mL MeOH and filered through a Millipore filter. Twenty microliters of the sample was injected into the HPLC.

2.3.2. Chromatographic conditions

For isoflavonoid assay, the mobile phase consisted of solvent A (acetonitrile) and solvent B (0.1% phosphoric acid, v/v) with a gradient elution (0–30 min, 22%–23% A; 30–40 min, 30%–35% A) The flow rate was 1 mL/min. The detection wavelength was set at 248 nm. A representative chromatogram of the isoflavonoid assay is shown in Figure 2. For astragaloside IV assay, the mobile phase consisted of solvent A (acetonitrile) and solvent B (water). An isocratic elution at 35% A was run. The flow rate was 0.8 mL/min. The drift tube temperature for ELSD was set at 102 ºC, and the nebulizing gas flow rate was 2.8 L/min. A representative chromatogram of astragaloside IV assay is shown in Figure 3.

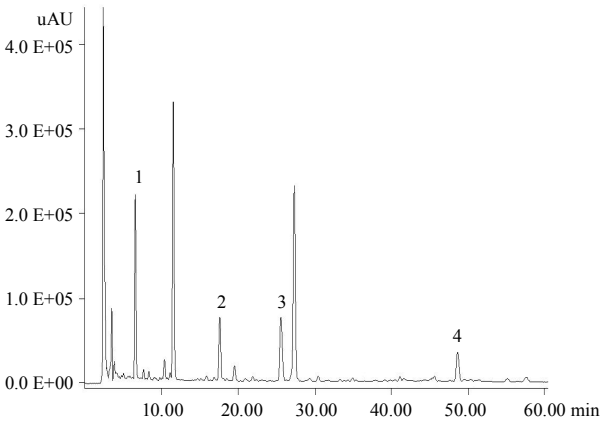

Figure 2. Representative HPLC-DAD chromatogram of the isoflavonoid assay.

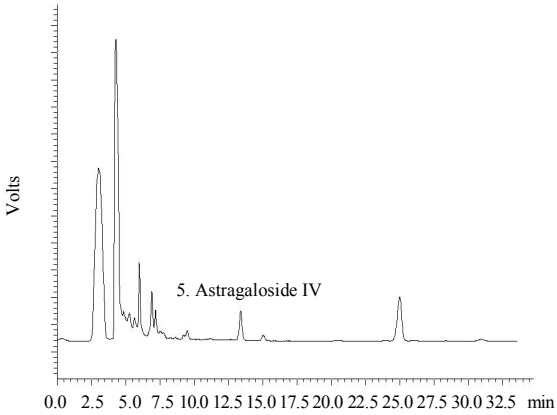

Figure 3. Representative HPLC-ELSD chromatogram of the astragaloside IV assay.

2.3.3. Calibration curve

Calibration was performed in the range of 1.6–320 mg/L using dilutions of the respective stock solutions. Calibration curves were generated by plotting the respective peak areas vs the concentrations (mg/L). Each calibration curve was obtained with 5–6 different concentrations in triplicate[15].

3. Results and discussion

The contents of calycosin-7-*O*-β-D-glucopyranoside (1), ononin (2), calycosin (3), formononetin (4) and astragaloside IV (5) were determined in 44 samples

of *A. membranaceus* var. *mongholicus* and 12 samples of *A. membranaceus* (Table 2)[17]. The amounts of calycosin-7-*O*-β-D-glucopyranoside and formononetin were higher than other compounds.

It also showed that the average contents of compounds 1, 2 and 5 in *A. membranaceus* var. *mongholicus* were consistently higher than those in *A. membranaceus* (Table 3).

Table 2. Contents of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin and astragaloside IV in RA (mg/g)

No.	Calycosin-7-*O*-β-D-glucoside	Ononin	Calycosin	Formononetin	Total isoflavonoids[a]	Astragaloside IV
1	0.033	0.013	0.021	0.007	0.073	0.010
2	0.026	0.012	0.011	0.004	0.053	0.010
3	0.012	0.006	0.006	0.002	0.026	0.011
4	0.012	0.006	0.006	0.002	0.026	0.011
5	0.010	0.012	0.003	0.002	0.027	*
6	0.032	0.021	0.002	0.003	0.058	0.012
7	0.051	0.021	0.014	0.004	0.091	0.031
8	0.013	0.008	0.009	0.003	0.034	0.021
9	0.010	0.005	0.015	0.008	0.039	0.019
10	0.015	0.008	0.015	0.005	0.043	0.007
11	0.015	0.006	0.032	0.013	0.065	0.013
12	0.012	0.003	0.028	0.014	0.058	0.007
13	0.015	0.006	0.031	0.017	0.070	0.006
14	0.018	0.007	0.028	0.017	0.070	0.008
15	0.015	0.006	0.008	0.003	0.032	0.007
16	0.026	0.015	0.019	0.007	0.068	0.005
17	0.023	0.012	0.017	0.005	0.056	0.008
18	0.013	0.005	0.007	0.003	0.028	0.008
19	0.015	0.010	0.003	0.002	0.029	0.024
20	0.019	0.010	0.008	0.003	0.040	0.029
21	0.013	0.008	0.023	0.011	0.055	0.007
22	0.004	0.001	0.006	0.001	0.013	0.005
23	0.010	0.005	0.010	0.004	0.028	0.011
24	0.036	0.012	0.011	0.003	0.062	0.017
25	0.018	0.006	0.019	0.008	0.051	0.006
26	0.003	0.001	0.001	0.000	0.004	0.008
27	0.020	0.007	0.011	0.002	0.040	0.017
28	0.007	0.005	0.007	0.006	0.025	*
29	0.022	0.011	0.007	0.002	0.042	0.025
30	0.013	0.006	0.006	0.002	0.026	0.013
31	0.018	0.010	0.017	0.006	0.051	0.010
32	0.015	0.012	0.026	0.017	0.070	0.009
33	0.008	0.002	0.017	0.007	0.034	0.009
34	0.022	0.005	0.007	0.001	0.035	0.006
35	0.010	0.003	0.032	0.010	0.055	0.007
36	0.009	0.003	0.004	0.001	0.017	0.011
37	0.014	0.011	0.005	0.003	0.033	0.007
38	0.009	0.005	0.007	0.004	0.026	*
39	0.023	0.018	0.007	0.005	0.053	0.008
40	0.007	0.002	0.013	0.007	0.029	*
41	0.013	0.007	0.008	0.004	0.032	*
42	0.015	0.008	0.006	0.002	0.030	0.014
43	0.011	0.006	0.005	0.002	0.025	0.007
44	0.016	0.007	0.006	0.002	0.032	0.007
45	0.005	0.001	0.003	0.001	0.011	*
46	0.005	0.001	0.001	*	0.008	*
47	0.004	0.001	0.002	0.001	0.008	0.006
48	0.010	0.003	0.011	0.003	0.028	0.007
49	0.010	0.006	0.009	0.003	0.028	0.009
50	0.014	0.008	0.006	0.003	0.030	0.012
51	0.017	0.005	0.018	0.004	0.044	0.010
52	0.013	0.006	0.002	0.006	0.027	0.006
53	0.003	0.002	0.025	0.008	0.039	0.008
54	0.013	0.003	0.027	0.006	0.048	0.012
55	0.006	0.005	0.005	0.002	0.017	0.003
56	0.019	0.004	0.051	0.014	0.088	0.010

[a]: Total isoflavonoids is the sum of the amounts of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, and formononetin.
*: Can be detected, but cannot be quantified.

Table 3. Average contents of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin and astragaloside IV in *A. membranaceus* var. *mongholicus* and *A. membranaceus* (mg/g)

Species source	Calycosin-7-*O*-β-D-glucoside	Ononin	Calycosin	Formononetin	Total isoflavonoids	Astragaloside IV
A. membranaceus var. *mongholicus*	0.016	0.008	0.012	0.005	0.042	0.012
A. membranaceus	0.010	0.004	0.013	0.005	0.031	0.008

Six samples (No. 1–2, 3–4 and 5–6) represented three groups of sources. In each group, the two samples were of spring and autumn collections obtained from the same cultivated place. The contents of compounds 1–5 collected in spring and in autumn were compared and the results are shown in Figure 4. The results demonstrated that the contents of isoflavonoids and astragaloside IV in RA harvested in spring were higher than those harvested in autumn. The reason may be that these chemical constituents could be accumulated during winter.

Twelve samples (No. 7–8, 9–4, 10–11, 12–13, 14–15 and 16–17) represented six groups of sources. In each group, the two samples are of different ages of plant collected in the same season and in the same cultivated places. The contents of compounds 1–5 in samples representing different ages of plants were compared and the results are shown in Figure 5. The results demonstrated that the contents of calycosin and formononetin in one-year plants were higher than those in two-year plants, the contents of calycosin-7-*O*-β-D-glucopyranoside and astragaloside IV in one-year plants were lower than those in two-year plants. There was no significant difference in the content of ononin in one-year plants and two-year plants.

Nine samples (No. 12–13–14, 15–16–17 and 18–19–20) represented three groups of sources. In each group, the three samples are of different commercial specifications in the herb market but same age of plants obtained from the same cultivated place. The contents of compounds 1–5 in samples representing different commercial specifications were compared and the results are shown in Figure 6. The results showed that there was no significant difference in the contents of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin and astragaloside IV in second-class sources and third-class sources. The contents of isoflavonoids and astragaloside IV in first-class sources were lower than those in second-class sources and third-class sources. It showed the contents of isoflavonoids and astragaloside IV in RA may not be related with the commercial specifications.

In our study, 56 RA samples obtained from different sources were collected, and the levels of calycosin-7-*O*-β-D-glucopyranoside, ononin, calycosin, formononetin and astragaloside IV were determined by HPLC for quality evaluation. The results showed that the contents of isoflavonoids and astragaloside IV varied significantly in different sources. The average contents of isoflavonoids and astragaloside IV in *A. membranaceus* var. *mongholicus* were consistently higher than those in *A. membranaceus*. The contents of total isoflavonoids and astragaloside IV were associated with the plant species, seasonal collections, ages and commercial specifications. Furthermore, one-year plants of *A. membranaceus* var. *mongholicus* harvested in spring have the highest contents of total isoflavonoids and astragaloside IV.

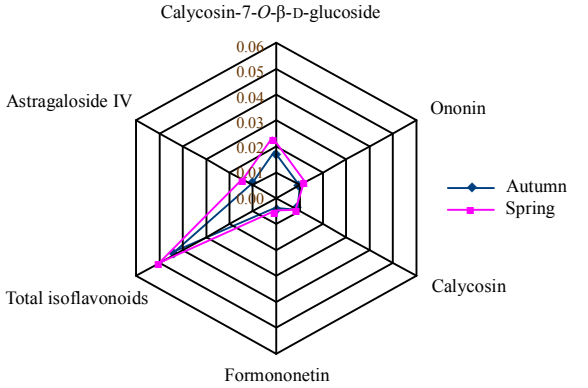

Figure 4. Comparison of the contents of compounds 1–5 collected in different seasons (mg/g).

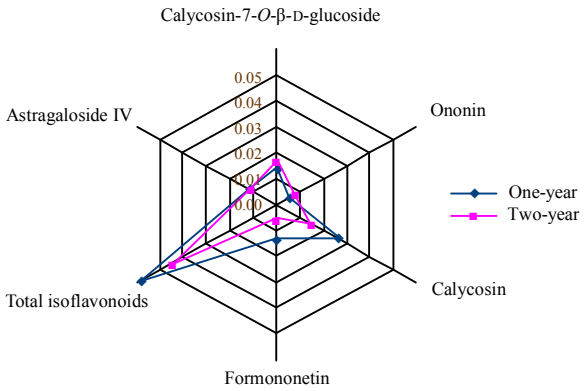

Figure 5. Comparison of the contents of compounds 1-5 in different ages of plants (mg/g).

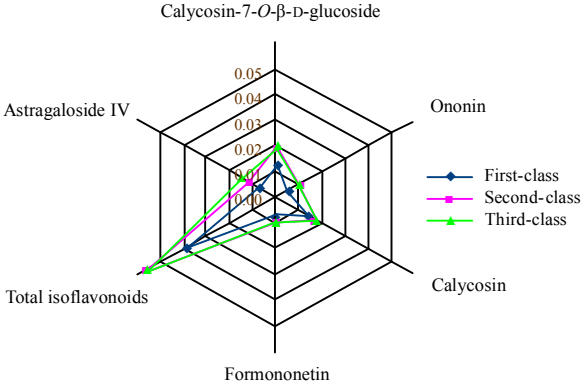

Figure 6. Comparison of the contents of compounds 1–5 in different commercial specifications (mg/g).

Acknowledgments

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不同来源中药黄芪的质量评价

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摘要: 中药黄芪为常用补气药, 市场上黄芪药材来源复杂, 质量参差不齐。本研究针对56份不同种源、不同栽培基地、不同年限、不同采收季节、不同商业规格的黄芪药材, 采用高效液相色谱法, 测定了其中的有效成分(毛蕊异黄酮、毛蕊异黄酮苷、芒柄花素、芒柄花苷及黄芪甲苷) 的含量, 对其进行质量评价。实验结果表明不同来源的黄芪药材的异黄酮类化合物及黄芪甲苷的含量存在明显的差异, 为黄芪药材的选用提供了一定的依据。

关键词: 黄芪; 质量评价; HPLC; 异黄酮类化合物; 黄芪甲苷