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Original article

Quality Evaluation of *Astragali Radix* based on DPPH Radical Scavenging Activity and Chemical Analysis

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ABSTRACT

Objective To assess the quality of *Astragali Radix* from different areas based on the biological evaluation and chemical analysis. **Methods** The bioassay method of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity for *Astragali Radix* was established. The parameters of DPPH assay including sample extraction time, reaction time, repeatability, and stability were detected. Furthermore, a method of HPLC-MS was developed to simultaneously determine calycosin-7-*O*-glucoside, ononin, formononetin, and astragaloside IV in *Astragali Radix* samples. And the total flavonoids and total saponins were detected by spectrophotometry. The relationship between DPPH evaluation and chemical analysis was studied by Pearson correlation analysis. **Results** Twelve batches of *Astragali Radix* from different origins showed a wide range of DPPH radical scavenging activities (IC_{50} = 1.395−9.894 μg/mL). Based on DPPH assay, Sample 10 derived from Inner Mongolia Autonomous Region (IC_{50} = 1.395 μg/mL) showed the best quality of all samples. Chemical analysis showed that different compounds selected as indices would cause different results for quality evaluation. Pearson correlation analysis revealed that the contents of total flavonoids (P = 0.032), calycosin-7-glucoside (P = 0.035), and astragaloside IV (P = 0.010) were positively correlated with DPPH radical scavenging activity. **Conclusion** Except for chemical analysis, DPPH radical scavenging activity can be used as a good alternative to assess and control the quality of *Astragali Radix*.

Key words

Astragali Radix; DPPH; HPLC-MS; quality evaluation

1. Introduction

Astragali Radix, named *Huangqi* in Chinese, is derived from the roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge. (Pharmacopoeia Committee of P. R. China, 2010). In traditional Chinese medicine (TCM), *Huangqi* is believed to have the function of reinforcing *qi* which is the vital energy of human body. And in clinic *Huangqi* tends to be used to strengthen the superficial resistance, induce urination, and

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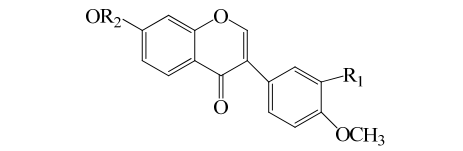
promote the growth of new tissue. Up to now, various biological activities of the compounds or extract from *Astragali Radix* have been reported such as immunomodulatory (Song et al, 2011), anticancer (Tian et al, 2012; Peng et al, 2011; Zhang et al, 2012), cardioprotective (Xu et al, 2008), antihyperglycemic (Chan et al, 2009), and hepatoprotective effects (Chien et al, 2011). The compounds contained in *Astragali Radix* have been isolated and identified as flavonoids, triterpene saponins, polysaccharides, and amino acids (Chu et al, 2010). Previous studies have been carried out on evaluating the quality of *Astragali Radix* by HPLC, in which the flavonoids, such as calycosin, calycosin-7-*O*-glucoside, ononin, formononetin, as well as astragalosides I, II, III, and IV, have been used as index components of the quality control of *Astragali Radix* (Wu et al, 2005; Xiao et al, 2004; Qi et al, 2009; 2006; Song et al, 2008).

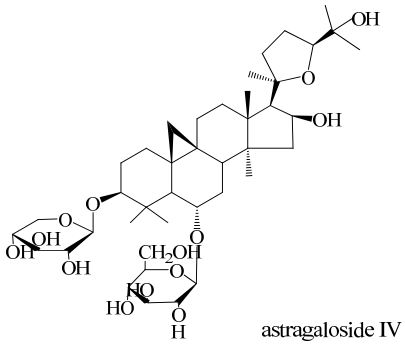
Currently, the quality of crude drug is mainly predicated on the analysis of one or more selected compounds. Ideally, these compounds should be responsible for the action and efficiency of the crude drugs. However, crude drugs consist of numerous compounds and their bioactivity can be considered to be due to the synergetic effects of several compounds (Ken et al, 2008). Therefore, to demonstrate the comprehensive properties of crude drugs, the development of a new evaluation strategy is required. For directly and comprehensively revealing the quality of Chinese materia medica (CMM), bioassay methods have proved to be effective (Li et al, 2009; Yan and Xiao, 2011).

Oxidative damage, induced by superfluous free radicals, was suggested to be the cause of aging, cancer, cardiovascular diseases, and neurodegenerative diseases (Stadtman, 1992) which were often treated by *Astragali Radix* in TCM. So in this study, a method based on anti-oxidantove activity *in vitro* of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging was established to assess the quality of *Astragali Radix*. DPPH is a stable free radical with an unpaired valence electron at one atom of nitrogen bridge (Eklund et al, 2005). The DPPH assay is based on a measurement of scavenging ability of anti-oxidants towards a free radical. The free radical DPPH, which shows absorption at 517 nm, is reduced to the corresponding hydrazine when it reacts with hydrogen donors. The decrease of the absorption can, therefore, represent the scavenging activity of the corresponding compound or crude extract. Although DPPH assay may be not as real as the method monitoring lipid peroxidation in a biological system, DPPH assay, as an easy and quick method, is suitable for the quality control of crude drug.

In previous studies, DPPH assay has been widely used to screen the compounds with anti-oxidantive activity. However except for the radical scavenger, DPPH radical scavenging activity is also influenced by reaction medium, reaction time, and the content of reaction systems (Sharma and Bhat, 1992) which were rarely discussed before. So in this study, the conditions of sample extracting, time of reaction, the repeatability and stability of the DPPH method for *Astragali Radix* were all discussed. Then 12 samples of *Astragali Radix* originated from different locations in China were investigated and compared. Furthermore in order to discover the differences and relations of DPPH assay and chemical analysis, the main compounds of calycosin-7-*O*-glucoside, ononin, formononetin, astragaloside IV, total saponins, and total flavonoids were also detected by the developed method of HPLC-MS and spectrophotometry (Figure 1). The analytical parameters of HPLC-MS such as selectivity, sensitivity, linear range, precision, and accuracy were all presented. And Pearson correlation coefficient was performed to reveal the relationship between DPPH radical scavenging activities and the detected compositions in *Astragali Radix*.



	R_1	R_2
calycosin 7-*O*-glucoside	OH	glucosyl
ononin	H	glucosyl
formononetin	H	H



astragaloside IV

Figure 1 Chemical structures of three flavonoids and one saponin detected by HPLC-MS

2. Materials and methods

2.1 Samples, reagents, and apparatus

Astragali Radix was collected from Hehuachi Herbal Drug Market in Chengdu city and Zhangshu Herbal Drug Market in Jiangxi province. The samples were authenticated by one of the authors, Prof. Mei-hong Fu, and were deposited in Institute of Chinese Materia Medica, China Academy of Chinese Medicine Sciences, Beijing, China. The related information is summarized in Table 1.

1,1-Diphenyl-2-picryl hydrazine (DPPH) was purchased from Sigma Aldrich Chemical Co. Calycosin-7-*O*-glucoside, ononin, formononetin, and astragaloside IV were purchased from National Institutes for Food and Drug Control (Beijing, China). The purity of each compound was higher than 98%. Acetonitrile was of HPLC grade from Merck (Darmstadt, Germany); Distilled water was further purified by Milli-Q system (Millipore, USA), formic acid was purchased from Chemical Company of Beijing (China), and other chemicals were of analytical grade.

Table 1 Information of *Astragali Radix*

Sample No.	Origins	Purchased from
1	Shouyang, Gansu province, China	Hehuachi Herbal Drug Market
2	Gansu province, China	Hehuachi Herbal Drug Market
3	Gansu province, China	Hehuachi Herbal Drug Market
4	Gansu province, China	Hehuachi Herbal Drug Market
5	Sichuan province, China	Hehuachi Herbal Drug Market
6	Longxi, Gansu province, China	Hehuachi Herbal Drug Market
7	Minxian, Gansu province, China	Hehuachi Herbal Drug Market
8	Gansu province, China	Zhangshu Herbal Drug Market
9	Litang, Sichuan province, China	Zhangshu Herbal Drug Market
10	Inner Mongolia Autonomous Region, China	Zhangshu Herbal Drug Market
11	Inner Mongolia Autonomous Region, China	Zhangshu Herbal Drug Market
12	Gansu province, China	Zhangshu Herbal Drug Market

Ultraviolet-visible spectroscopy was performed on a UV−2000 Spectrophotometer (Unico Instrument Co., Ltd., China). LC-MS analysis was performed on an Agilent 1100 Series LC System equipped with a single Quadrupole MS.

2.2 Preparation of standards

The solution of DPPH was prepared by dissolving 8.40 mg in 100 mL measuring flask with methanol. The content was 0.084 mg/mL (0.213 mmol/L). The standard stock solutions of calycosin-7-*O*-glucoside, ononin, formononetin, and astragaloside IV were prepared by dissolving 5 mg of each compound in a 25 mL measuring flask with methanol. Working standard solutions containing each of the four compounds were prepared by diluting the stock solutions with methanol to a series of proper concentration (60−0.2 μg/mL) for HPLC-MS analysis. The stock solutions of rutin and astragaloside IV were prepared by dissolving 2 mg compound in a 10 mL measuring flask, respectively. Then the stock solutions of rutin and astragaloside IV were diluted with methanol to a series of proper concentration for analysis on total saponins and total flavonoids.

2.3 Preparation of herb extract

The dried roots were powdered to a homogeneous size by a mill, sieved through a No. 40 mesh (diameter of 380 μm), and further dried at 60 °C in the oven for 2 h to constant weight. The powder sample accurately weighed (0.4 g) was added to a flat-bottomed flask containing 40 mL methanol. The mixture was weighed and then heated under reflux for 1 h. Then the extract was cooled and the weight was complemented with methanol. After filtered, the extracts were stored at −20 °C until use. Before DPPH assay, the extract of the sample was diluted with methanol to a series of proper concentration. Before HPLC and spectrophotometry analysis, the extract of every sample was filtered through 0.45 μm filter.

2.4 DPPH free radical scavenging assay for Astragali Radix

DPPH radical scavenging assay for the determination of *Astragali Radix* was based on the previous study with modifications (Brand-Williams et al, 1995; Manjaly et al, 2012; Alma et al, 2003). Briefly, 1.0 mL samples of various concentration in methanol were added directly to 1.0 mL of DPPH solution in methanol (0.084 mg/mL) and then immediately shaken thoroughly. The absorbance (*A*) was measured at 517 nm after 40 min under 25 °C, and the anti-oxidant capability (*AA*) was expressed as the percentage of DPPH reduced, which was calculated with the following formula.

$$AA_{\mathrm{DPPH}} = A_{\mathrm{B}} - A_{\mathrm{S}} / A_{\mathrm{B}}$$

Where A_{S} is the absorbance of DPPH solution after reacting with the sample at a given concentration and A_{B} is the absorbance of DPPH solution with a methanol blank instead of the sample.

The percentage of DPPH reduced was plotted against the concentration of each sample, and an IC_{50} value, which was defined as the concentration of the sample needed to scavenge 50% of the DPPH, was calculated from the graph. Because the range of accuracy for spectrophotometric measurement should be within an absorbance of 0.2−0.8 (Ayres, 1949), pre-experiments were conducted to confirm the proper contents for measurement.

The method parameters of DPPH radical scavenging activity for *Astragali Radix* including the reaction time, sample extracting time, repeatability, and stability were discussed as follows: Real-time absorbance of one prepared sample was recorded at room temperature (25 °C). The reaction time was decided when the absorbance became stable. A series of prepared samples with different extracting time were tested. The optimal extracting time was decided based on absorbance. Stability was tested with one prepared sample at room temperature and analyzed at 0, 2, 4, 8, 12, and 24 h within 1 d, respectively. The relative standard deviation (RSD) of absorbance was taken as the measures of stability. Six samples of parallel preparation were tested. The RSD of absorbance was taken as the measurement of repeatability.

2.5 Determination of total saponins and total flavonoids contents

Saponins and flavonoids are two major types of active compounds responsible for the biological activities of

Astragali Radix, so the total saponins content and total flavonoids content in *Astragali Radix* were measured. According to Jia et al (1999), the content of total flavonoids was determined as follows: The sample (8 mL) was mixed with 0.3 mL $NaNO_2$ solution (0.05 g/mL) in a graduated test tube and incubated for 5 min, and then 0.3 mL $Al(NO_3)_3$ solution (0.1 g/mL) was added and incubated for 6 min. The reaction was terminated by adding 4 mL NaOH solution (1 mol/L) and then dilute with 95% ethanol to 13 mL. *A* of the mixture was determined immediately at 504 nm. The content of total flavonoids was expressed as rutin equivalents (mg RE/g).

The content of total saponins was measured using the method of vanillin-glacial acetic acid and perchloric acid (Li et al, 2008; Oleszek, 2002). Firstly, 0.2 mL sample was placed into glass tube and held in water bath at 90 °C until drying. Secondly, 0.2 mL vanillin-glacial acetic acid solution (5%) and 0.8 mL perchloric acid solution were added and mixed. Thirdly, the closed glass tube with plug was heated in 70 °C water for 15 min. After cooling, 5 mL glacial acetic acid was added and mixed. And then the absorbance of sample was measured at 471 nm at room temperature. The results were expressed as astragaloside IV equivalents (mg Ast/g).

2.6 HPLC-MS analysis

Chromatographic separation was carried out at 35 °C on an Agilent Zorbax Eclipse Plus C_{18} (150 mm × 4.60 mm, 4 μm) column. The mobile phase consisted of 0.5% formic acid water (A) and methanol (B) using a gradient elution of 30%−65% B at 0−10 min, 65% B at 10−40 min, and 65%−30% B at 40−41 min. The injected volume of samples and working standard solutions was 5 μL. The flow rate was 0.5 mL/min and 50% eluent at the column outlet flow into the mass spectrometer.

Detection was performed by an Agilent Single-quadrupole Detector equipped with ESI source. The mass analysis was carried out in negative mode under selected ion monitoring (SIM). The detected ions of flavonoids and saponins are shown in Table 2. Conditions of ESI source were as follows: drying gas (N_2) flow rate, 9.0 L/min; drying gas temperature, 350 °C; nebulizer, 0.2 MPa; capillary voltage, 3500 V.

Method validation was examined for assay precision and linearity; The limits of quantification (LOQ), limits of detection (LOD), stability, repeatability, and accuracy under the HPLC analytical conditions were described above. The calibration curve of each compound was performed with at least five kinds of appropriate concentration. The LOD and LOQ under the present chromatographic conditions were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. The intra- and inter-day precision was determined by analyzing a standard mixture solution of the four standards for six times during a single day and on three consecutive days. Stability was tested with one prepared sample at room temperature and analyzed at 0, 2, 4, 8, 12, 24, and 48 h within 2 d. The RSD of peak area was taken as the measures of stability. Six samples of parallel preparation were tested. The RSD of the content of compounds was taken in the measurement of repeatability. The accuracy was evaluated by recovery test. The six samples were prepared by adding known amounts of standards to *Astragali Radix* extracts, which were then analyzed by HPLC.

Table 2 Selected ion monitoring for analytes in negative mode

Analytes	MW	Retention time /min	Monitoring ions	Experimental mass (*m/z*)
calycosin-7-glucoside	446.40	6.92	$[M + HCOO^-]^-$	491.00
ononin	430.40	10.31	$[M + HCOO^-]^-$	475.10
formononetin	268.26	15.10	$[M - H]^-$	267.10
astragaloside IV	784.97	36.10	$[M + HCOO^-]^-$	829.30

2.7 Statistical analysis

In order to establish and confirm the correlation between anti-oxidant capacity (*Y*) and contents of chemical components (*X*) in *Astragali Radix*, we used the Pearson correlation coefficient to evaluate the correlation degree for *Y* and *X*. Pearson correlation coefficient is sensitive to a linear relationship between two variables which may exist even if one is a nonlinear function of the other. It is obtained by dividing the covariance of the two variables by the product of their standard deviations as below.

$$P_{X,Y} = corr(X,Y) = \frac{\mathrm{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E[(X-\mu_X)(Y-\mu_Y)]}{\sigma_X \sigma_Y}$$

Where μ_X and μ_Y are expected values, σ_X and σ_Y are standard deviations for *X* and *Y*, *E* is the expected value operator, and cov means covariance.

Besides, the significances for the correlation coefficients were calculated to show how unlikely a given correlation coefficient would have no relationship in the population.

3. Results and discussion

3.1 DPPH free radical scavenging ability of samples

From the chart of *A*-time (Figure 2A), we could see that after 40 min of reaction, the absorption level stabilize. Hence, the reaction time was decided at 40 min. Based on the optimal reaction time, one sample extracted with different time of 0.5, 1.0, 1.5, 2.0, and 2.5 h were analyzed respectively. As shown in Figure 2B, 1 h was chosen as the sample extracting time in consideration of the radical scavenging ability. Stability was tested with one prepared sample which was analyzed at 0, 2, 4, 8, 12, and 24 h after thawing respectively. The RSD of *A* was 1.34%, which illustrated that within 24 h after thawing, the sample for DPPH free radical scavenging was stable. Six samples of parallel preparation were tested. The RSD of *A*, as the measurement of repeatability, was 4.34%.

Based on the established method of DPPH assay, 12 batches of samples which originated from various districts and vitamin C (VC) were analyzed. VC as a positive drug showed obvious activity. The IC_{50} of VC was 0.01 mg/mL in the above reaction system. As shown in Table 3, the IC_{50} of all samples had a wide range from 1.395 to 9.894 mg (crude drug)/mL. Within all the collected samples, the Samples 10 and 11 which originated from Inner Mongolia Autonomous Region showed the best quality. IC_{50} of Sample 10, with the highest activity, was 1.395 mg (crude drug)/mL. IC_{50} of Sample 11 was 1.927 mg (crude drug)/mL. While IC_{50} of Sample 9, with the lowest activity, was 9.894 mg (crude drug)/mL. The results indicated under the established systems, DPPH assay could be used to evaluate the quality of *Astragali Radix* directly and effectively (Table 3).

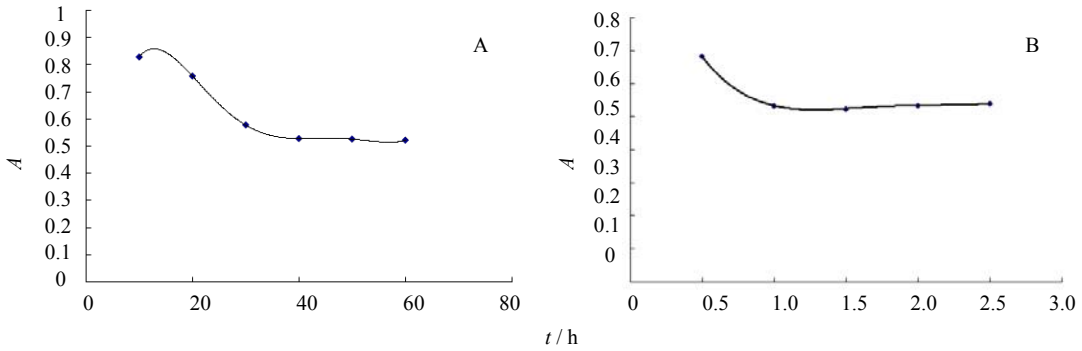


Figure 2 Determination of optimal reaction time (A) and sample extracting time (B)

Table 3 Anti-oxidantive activity IC_{50} and contents of detected compounds

Sample No.	IC_{50} / (mg·mL^{-1})	Total flavonoids / (mg·g^{-1})	Total saponins / (mg·g^{-1})	Calycosin-7-*O*-glucoside / (mg·g^{-1})	Ononin / (mg·g^{-1})	Formononetin / (mg·g^{-1})	Astragaloside IV / (mg·g^{-1})
1	3.049	1.32	4.56	0.480	0.216	0.051	0.038
2	3.482	0.80	5.35	0.370	0.124	0.079	0.055
3	4.360	0.67	6.78	0.373	0.104	0.099	0.020
4	3.199	0.82	3.45	0.360	0.093	0.104	0.052
5	4.369	0.61	2.43	0.249	0.047	0.113	0.014
6	2.777	0.72	2.67	0.297	0.059	0.144	0.037
7	2.109	1.23	5.67	0.590	0.160	0.065	0.062
8	5.621	0.77	5.77	0.318	0.054	0.117	0.031
9	9.894	0.28	1.34	0.037	0.000	0.014	0.010
10	1.395	4.94	6.78	0.393	0.078	0.105	0.084
11	1.927	5.87	6.88	0.701	0.140	0.223	0.030
12	2.723	0.63	2.13	0.409	0.134	0.054	0.059

3.2 Contents of total saponins and total flavonoids

From the spectra of full wavelength scan (Figure 3), we can see that the common maximum peak of the prepared sample and the standard substance were at 471 and 504 nm for total saponins and total flavonoids respectively. So 471 and 504 nm were decided as the detection wavelengths. As shown in Table 3, the contents of total saponins in all samples were 1.34−6.88 mg Ast/g. The contents of total saponins in Sample 3, 10, and 11 were 6.78, 6.78, and 6.88 mg Ast/g which were obviously higher than those in other samples. The content range of total flavonoids was from 0.28 to 5.87 mg RE/g. The content of total flavonoids in Sample 11 was 5.87 mg RE/g, which was the highest of all. The contents of total flavonoids and total saponins in Sample 9 were 0.28 mg RE/g and 1.34 mg Ast/g, which were both the lowest of all. The results of quality evaluation based on the contents of total saponins and total flavonoids were similar with the results of DPPH assay.

3.3 Method validation of HPLC-MS analysis

Representative HPLC-MS of the sample and the standards is shown in Figure 4. The equations, linear ranges, LOD, and LOQ for the three flavonoids and one saponin detected are summarized in Table 4. The RSD values of intra- and inter-day precision were 0.13%−2.01% and 0.98%−2.76% respectively (Table 5). Six parallel prepared samples were analyzed to confirm the repeatability and the RSD of repeatability was 1.3%−2.2%. To determine the recovery, the contents of the four analytes in a sample were calculated according to their respective calibration curves. The same volume of each analyte presented in the sample was spiked into the sample for six times. Then the fortified samples were extracted, disposed as described above and analyzed with the procedure. The average recoveries were estimated by the formula: recovery = (amount found − original amount) / amount spiked. The recovery ranged from 94.7% to 99.3% and the RSD was from 1.9% to 5.5% (Table 6).

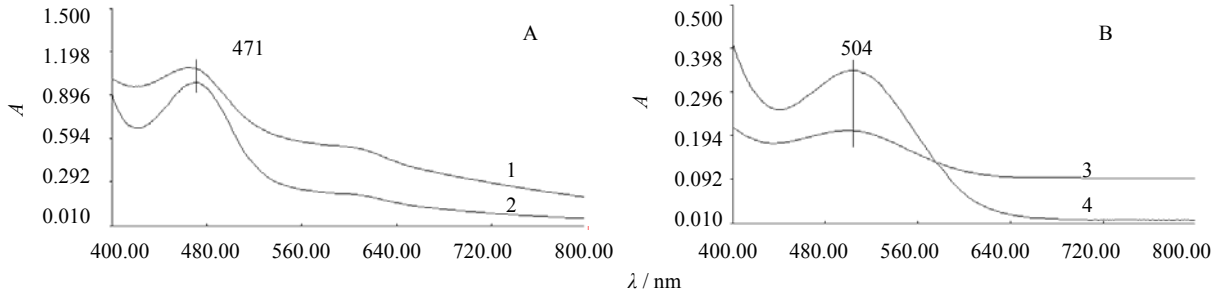


Figure 3 Spectra of total saponins (A) and total flavonoids (B) by full wavelength scan (400.00−800.00 nm)

1: prepared sample 2: astragaloside IV 3: rutin 4: prepared sample

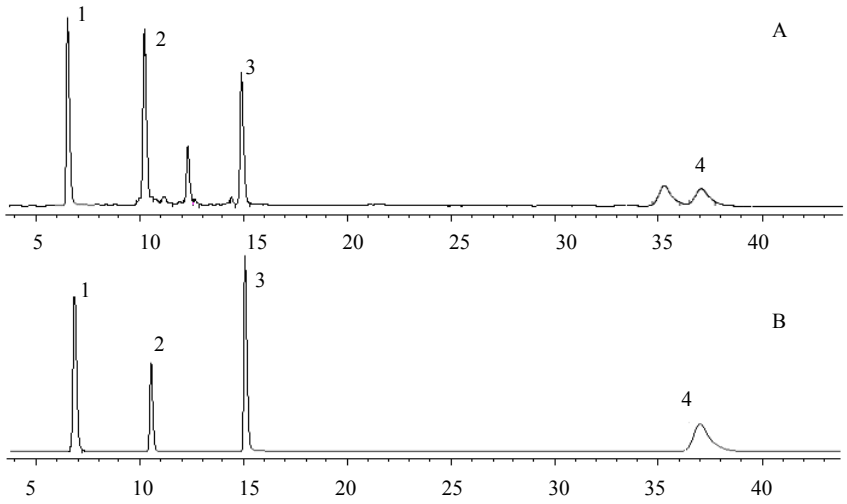


Figure 4 Representative HPLC-MS (selected ion monitoring) of analytes (A) and prepared sample (B)

1: calycosin-7-*O*-glucoside 2: ononin 3: formononetin 4: astragaloside IV

Table 4 Regression data, LODs, and LOQs of three flavonoids and one saponin

No.	Analytes	Regression equations	r^2	Linear ranges / (μg·mL^{-1})	LOD / ng	LOQ / ng
1	calycosin-7-*O*-glucoside	Y = 58 036X − 448.44	0.9993	0.224−11.20	0.37	1.12
2	ononin	Y = 99 691X + 12 722	0.9986	0.175− 8.750	0.44	0.875
3	formononetin	Y = 202 400X + 55 841	0.9935	0.248− 4.960	0.41	1.24
4	astragaloside IV	Y = 95 996X + 21 892	0.9995	0.302−30.20	0.76	1.51

Table 5 Precision and repeatability of four analytes

No.	Analytes	Precision				Repeatability (n = 6)	
		Intra-day (n = 6)		Inter-day (n = 18)		Mean / (mg·g^{-1})	RSD / %
		Mean / (μg·mL^{-1})	RSD / %	Mean / (μg·mL^{-1})	RSD / %		
1	calycosin-7-*O*-glucoside	11.2	1.86	11.4	2.21	0.409	2.2
2	ononin	8.7	0.68	8.8	1.03	0.134	1.9
3	formononetin	4.9	0.13	5.0	0.98	0.054	1.3
4	astragaloside IV	30.2	2.01	30.4	2.76	0.059	3.5

3.4 Contents of calycosin-7-glucosid, ononin, formononetin, and astragaloside IV

In Table 3, the content ranges of calycosin-7-*O*-glucoside, ononin, formononetin, and astragaloside IV fell in 0.037−0.590, 0−0.126, 0.014−0.223, and 0.010−0.084 mg/g, respectively. Among the three flavonoids, calycosin-7-*O*-glucoside was the most abundant. The content of calycosin-7-*O*-glucoside in Sample 7, which originated from Minxian county of Gansu province, was 0.590 mg/g and higher than any others. The content of calycosin-7-*O*-glucoside in Sample 9 was 0.037 mg/g and was the lowest. The content of ononin

Table 6 Recovery of compounds determined by standard addition method (n = 6)

Analytes	Sample weight / g	Original amount / μg	Added amount / μg	Detected amount / μg	Recovery rates / %	RSD / %
calycosin-7-*O*-glucoside	0.20	78.6	85.7	163.7	99.3	5.5
ononin	0.20	15.6	15.2	30.0	94.7	1.9
formononetin	0.20	21	18.8	39.6	98.8	3.8
astragaloside IV	0.20	16.8	16.4	32.9	98.2	3.6

in Sample 1, which originated from Shouyang county of Gansu province, was 0.216 mg/g and was the highest, while in Sample 9 ononin was undetected. Regarding to formononetin, the highest content was found in Sample 11 (0.223 mg/g) and the lowest was found in Sample 9 (0.014 mg/g). When astragaloside IV was chosen as indices for quality evaluation, Sample 10 (0.084 mg/g) showed the best quality and Sample 9 (0.010 mg/g) displayed the worst quality.

From the results above, we can see that the different compounds selected as indices for quality evaluation may result in different outcomes. In order to compare the outcomes between DPPH assay and chemical analysis assay, Pearson correlation analysis was performed.

3.5 Correlation analysis of DPPH scavenging activity and saponin / flavonoid contents

Based on the Pearson correlation analysis, the correlation between DPPH scavenging activity (Y) and saponin/flavonoid contents (X) was analyzed. From Table 7 we can see that X_6 is the most correlated factor with a satisfactory significance of 0.010. Except for X_6, X_1 (P = 0.032) and X_3 (P = 0.035) are also significant variables with relatively high correlation coefficients which can be taken into consideration as the correlated factors for Y. Consequently, among all of the six variables, X_1, X_3, and X_6, which represent total flavonoids, calycosin-7-*O*-glucoside, and astragaloside IV, may have notable influences on the Y value. And the contents of total flavonoids, calycosin-7-*O*-glucoside, and astragaloside IV are higher, the IC_{50} will be smaller.

Table 7 Pearson correlation coefficient for IC_{50} (Y, mg/mL) and contents of detected compounds (X_1−X_6 , mg/g)

Compounds	Pearson correlation coefficient for Y and X	Significance (2-tailed)
X_1	−0.620*	0.032
X_2	−0.315	0.318
X_3	−0.610*	0.035
X_4	−0.502	0.097
X_5	−0.172	0.593
X_6	−0.706*	0.010

* means the coefficient is significant at the 0.05 level

X_1: total flavonoids X_2: total saponins X_3: calycosin-7-glucoside X_4: ononin X_5: formononetin X_6: astragaloside IV

4. Conclusion

In this study, to assess the quality of *Astragali Radix* directly and comprehensively, the bioassay method of DPPH radical scavenging activity for *Astragali Radix* is developed. The parameters of DPPH assay including sample extraction time, reaction time, repeatability, and stability are all discussed. Based on the established method of DPPH assay, 12 batches of samples originated from various districts are analyzed. The IC_{50} of all samples has a wide range from 1.395 to 9.894 mg (crude drug)/mL. Within all the collected samples, the Samples 10 and 11 which originated from Inner Mongolia Autonomous Region show the best quality. The results indicate DPPH assay could be used to evaluate the quality of *Astragali Radix* directly and effectively.

Secondly, in order to compare the outcomes of DPPH assay and chemical analysis, calycosin-7-*O*-glucoside, ononin, formononetin, astragaloside IV, total saponins, and total flavonoids in *Astragali Radix* are detected by the developed method of HPLC-MS and spectrophotometry. The precision and accuracy of the HPLC-MS method are confirmed through intra- and inter-day validation and the results of a recovery test. Using this HPLC-MS method, calycosin-7-*O*-glucoside, ononin, formononetin, and astragaloside IV in *Astragali Radix* have a good separation and are simultaneously determined. The total saponins and total flavonoids are analyzed according to the previously reports by spectrophotometry. From the results, we can see that the different compounds selected as indices for quality evaluation result in different outcomes. Pearson correlation analysis shows the contents of total flavonoids (P = 0.032), calycosin-7-*O*-glucoside (P = 0.035), and astragaloside IV (P = 0.010) are positive correlated with the DPPH radical scavenging activity. In conclusion, except for chemical analysis, DPPH radical scavenging activity can be used as a good alternative to assess and control the quality of *Astragali Radix*.

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