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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 399–406
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Chemical comparison and classification of Radix Astragali by determination of isoflavonoids and astragalosides

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Received 31 August 2007; received in revised form 15 December 2007; accepted 20 December 2007
Available online 31 December 2007

Abstract

Eleven major isoflavonoids and three major astragalosides in the xylem and bark of cultivated Radix Astragali (RA) from different cultivated regions of China were determined by high performance liquid chromatography. The results showed that the contents of astragalosides in the bark are up to 74-fold higher than in the xylem, and that thin roots contained more astragalosides than thick roots. Although the contents of isoflavonoids varied between samples, no significant difference was observed between the isoflavonoids content of xylem and bark, or between that of thin and thick roots. It was also found that the chemical profile of isoflavonoids in the xylem and bark are related to their cultivated regions. Constituents in either xylem or bark were divided into five groups according to their chemical structures: (1) Group 1 (**G1**), contained calycosin and related constituents; (2) Group 2 (**G2**), contained ononin and related constituents; (3) Group 3 (**G3**), contained (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan and related constituents; (4) Group 4 (**G4**), contained (3*R*)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan and related constituents; and (5) Group 5 (**G5**), contained astragalosides, compounds **AG I**, **AG II**, and **AG IV**. Based on the integrated contents of constituents in each group, the cultivated region of RA was successfully distinguished by principal components analysis (PCA). Chemical constituents in RA cultivated from different regions of China were compared and it was concluded that the quality of thin RA roots is better than thick RA roots.
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Keywords: Radix Astragali; High performance liquid chromatography; Principal component analysis; Isoflavonoids; Astragalosides

1. Introduction

Radix Astragali (RA), known as Huangqi in China, is the dried root of *Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* var. *Mongholicus* (Bge.) Hsiao (family Leguminosae). RA has been shown to have immunostimulant, tonic, hepatoprotective, diuretic, antidiabetic analgesic, expectorant, and sedative properties [1–4], and is commonly used in traditional Chinese medicine for treatment of many diseases. In addition to its medicinal use, it is also used in nutraceutical products, including herbal teas, soft drinks, soups, and trail mixes [2,5]. Since the quality of RA raw material is not only the prerequisite for its medical and nutraceutical effects, but also an important determinant of costs, it has attracted increasing attention.

Following the guidelines of the Good Agriculture Practice (GAP) in China, most RAs nowadays are from cultivated *A. membranaceus* var. *Mongholicus* (Bge.) Hsiao. Adulterant species of genus *Astragalus* L. are seldom found in the market. Even so, the quality of RA is still questionable since it is different among cultivated regions [6,7]. Furthermore, the criteria for quality assessment are different between the pharmacopoeia, publications and commercial market. In the pharmacopoeia and publications, selected active constituents in RA such as astragalosides and isoflavonoids are used as standards for the quality control: high content of active constituents indicates high quality [6–13]. In the market, RA is graded by the root length, diameter, and physical appearance: the longer and thicker the root, the higher the quality [12,14]. In addition, the price is different between cultivated cultivating regions; the highest priced RA produced in Shanxi Province is over 30-fold higher than that of the lowest priced RA. Previous studies on *Astragali* species with HPLC, LC–MS are well documented [6–12,15–18], however there is a limited amount of information available on the complete quality assessment of RA since most publications focused

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doi:10.1016/j.jpba.2007.12.036

on the analytical methodological study and genuine authentication. Thus, a standardized method is needed to fully assess the quality of cultivated RA. The objective of our study is to accomplish this using a combination of principle component analysis (PCA) and HPLC.

PCA allows both the identification of the most important directions of variability in a multivariate data matrix and the multivariate classification of the analyzed samples. In conjunction with HPLC data, PCA has been applied to quality studies of natural products [19–23]. However, for the very first time, our lab was able to use PCA to distinguish the RAs from different cultivated regions.

HPLC was also used to analyze the constituents in the xylem and bark of RA from different cultivated sites of China. Detailed HPLC quantitative analysis results were employed for its quality evaluation.

2. Experimental

2.1. Plant materials

All samples were taken from the root of *A. membranaceus* var. *Mongholicus* (Bge.) Hsiao. They were collected in September and October of 2005, from the cultivated farms in Inner Mongolia, Shanxi, Gansu and Heilongjiang provinces of China. Roots of RA in each sample population were randomly divided into three to five groups. Each group contained at least five roots. Branch roots were separated from the taproots. After peeled off the bark, the diameter of xylem and thickness of bark at the upper 1/3, the middle part and the lower 1/3 of a root were measured. The taproots or branch roots from at least five plants of the same population were combined, weighed and ground into fine powder, respectively. The collected powder was stored in dry conditions until further use. The voucher specimens were deposited in the Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China.

2.2. Chromatography

HPLC analysis was carried out according to our previous published method [8]. Briefly, quantitative analyses were performed on an Agilent 1100 HPLC system with DAD and Alltech ELSD 2000ES (Alltech, USA) detectors. An XTerra® MS C_8 column (150 mm × 2.1 mm; 3.5 μm, Waters, USA) together with an Xterra® C_8 guard column was used for the separation. The mobile phase consisted of (A) 0.2% TFA and (B) acetonitrile with a gradient elution of 88–70–15–0% of A and 12–30–85–100% of B at 0–30–50–55 min. The column was thermostated at 40 °C and the flow rate was 0.3 mL/min. The UV detection wavelengths were set at 210 nm. The ELSD detection was set at tube temperature at 100 °C and gas (N_2) flow rate at 1.5 mL/min.

2.3. Chemicals and reagents

The authentic reference compounds of ononin (99.9%), formononetin (>99.0%), astragaloside I (>98.0), and astragaloside II (>98.0%) were purchased from ChromaDex (Santa Ana, USA). Astragaloside IV (>98.0%) was purchased from the Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Other reference compounds including calycosin-7-*O*-D-glucopyranpside, calycosin and (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside were isolated and identified according to the published methods [8]. Purity of these isolated reference compounds was assayed to be 98.0% by the proposed HPLC-UV-ELSD method. Methanol of HPLC grade was purchased from Fisher Scientific (Loughborough, UK). Acetonitrile of HPLC grade was obtained from Merck (Darmstadt, Germany). Water was prepared by a Mill-Q purification system from Millipore (Milford, MA, USA). All other chemicals are analytical grade.

2.4. Sample preparation

The sample preparation was performed according to our previously published method with minor modification [8]. For isoflavonoids and astragalosides analysis, 0.5 g fine powder was extracted twice with 20 mL of methanol by ultrasonication for 30 min. The combined methanol extract was filtered and evaporated to dryness. The residue was reconstituted in 2 mL of methanol and filtered before injection. 2 μL of the sample was injected to HPLC for the determination of isoflavonoids and astragalosides.

2.5. Statistics

Statistical analysis was performed using SPSS for Windows version 14.0 (SPSS Inc., Chicago, IL, USA). The difference between xylem and bark was evaluated using one-way analysis of variance (ANOVA). It is considered to be statistical significant when P value is less than 0.05. The data of isoflavonoids and astragalosides contents were analyzed using factor analysis with principal components extraction. Regression method was used to calculate the factor scores.

3. Results and discussion

3.1. Calibration curves, limits of detection and quantification, precision and recovery

Reference standards of calycosin-7-*O*-β-D-glucoside (**1**), ononin (**3**), (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside (**4**), calycosin (**6**), formononetin (**9**), astragaloside I (**AG I**), astragaloside II (**AG II**), and astragaloside IV (**AG IV**) were accurately weighed, dissolved in methanol to form the stock solutions with the concentration of each compound being 0.636 mg/mL (**1**), 0.574 mg/mL (**3**), 0.550 mg/mL (**4**), 0.382 mg/mL (**6**), 0.500 mg/mL (**9**), 0.302 mg/mL (**AG I**), 0.642 mg/mL (**AG II**), and 0.506 mg/mL (**AG IV**). The stock solution was diluted with methanol in a volumetric flask to give serial concentrations for the calibration curves. The regression equations for compounds **1**, **3**, **4**, **6**, and **9** were calculated in the form of $y = bx + a$, while the regression equations for the three saponins (**AG I**, **AG II**, and **AG IV**) were

determined by ELSD and described as $\log y = b\log x + a$, where y and x were peak area and amount of compound injection. The limit of detection (LOD) and limit of quantification (LOQ) were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively. The results of calibration, LOD and LOQ are shown in Table 1.

The intra-day precision was evaluated by determining a standard mixture solution of the seven markers under the optimized condition six times a day. For inter-day precision, the measurement was conducted two times per day for 3 consecutive days. As shown in Table 1, the intra-day and inter-day relative standard deviations (R.S.D.s) were 0.7–2.6 and 0.5–3.8% respectively.

The recovery of the eight marker components from RA was determined by spiking known amount of reference standards to 0.25 g of RA (Gansu, MixedR) with known contents of the eight markers. The spiked samples were then treated as described in Section 2.4. The percent of recovery ranged from 95.5 to 104.3%.

3.2. Physical appearance and measurement of samples

Samples from Shanxi province were cultivated for 5–10 years, while those from Gansu, Heilongjiang and Inner Mongolia were cultivated for 2–3 years. The average diameter within the same sample population was reported (Table 2). The diameter of the xylem ranged from 1.7 to 16.5 mm, while the thickness of the bark ranged from 1.4 to 3.8 mm. The weight ratio between bark and xylem ranged from 0.6 to 4.0. It was shown that, as the diameter of the xylem increased, the thickness of bark slightly increased, whereas the weight ratio between bark and xylem increased significantly (data not shown).

3.3. Chemical profile and quantitative analysis

Fig. 1 showed typical chromatograms of extracts from bark and xylem of RA. Eleven major isoflavonoids namely, calycosin-7-*O*-β-D-glucoside (**1**), calycosin-7-*O*-β-D-glucoside-6″-*O*-malonate (**2**), ononin (**3**), (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside (**4**), (3*R*)-7,29-dihydroxy-3′,4′-dimethoxyisoflavan-7-*O*-β-D-glucoside (**5**), calycosin (**6**), formononetin-7-*O*-β-D-glucoside-6″-*O*-malonate (**7**), astrapterocarpanglucoside-6″-*O*-malonate (**8**), formononetin (**9**), (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan (**10**), (3*R*)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan (**11**), were identified by LC–MS/MS, and three astragalosides namely, astragaloside I (**AG I**), astragaloside II (**AG II**), and astragaloside IV (**AG IV**) were identified by spiking the known concentration of reference standards. Chemical structures of theses compounds are shown in Fig. 2.

3.4. Contents of astragalosides and isoflavonoids

The efficiency of different extraction methods and parameters, which affected the extractions was evaluated in our previous study [8]. In this study, ultrasonic extraction, which is a simple and efficient method was used for the sample preparation.

Table 1
Regression equation, correlation coefficients, linearity ranges, recovery and limit of detection (LOD) and limit of quantitation (LOQ) for the markers of RA

Compound	Linearity			LOD (ng)	LOQ (ng)	Recovery			Precision	
	Regression equation	Range (μg/mL)	R^2			Added (μg)	Detected (μg)	Yield (%)	Inter-day ($n=3$)	Intra-day ($n=6$)
1	$y=57.25x+44.45$	3.2–159	0.9998	1.0	3.4	79.5	81.7	102.8 ± 1.2	3.0	2.6
3	$y=57.62x+65.25$	7.2–144	0.9998	1.7	5.5	71.5	68.6	95.9 ± 1.4	1.2	1.7
4	$y=140.49x+22.39$	2.8–110	0.9998	4.1	13.7	55.0	53.0	96.3 ± 0.3	0.5	0.7
6	$y=11.06x-265.54$	2.5–100	0.9998	1.8	6.1	50.0	50.1	100.2 ± 0.7	2.7	2.6
9	$y=10.80x-3.46$	4.8–191	0.9998	2.1	7.0	95.5	97.4	102.0 ± 2.6	2.5	1.5
AG I	$y=1.65x-0.04$	11.5–115	0.9987	12.3	41.1	151.0	157.0	104.3 ± 1.9	0.7	2.4
AG II	$y=1.69x+0.17$	15.6–156	0.9986	8.7	28.9	78.0	77.8	99.7 ± 2.2	2.6	2.2
AG IV	$y=1.66x+0.37$	15.1–302	0.9996	8.9	29.6	57.5	54.9	95.5 ± 0.5	3.8	2.5

Table 2
Comparison of contents of isoflavonoids and astragalosides between xylem and bark of RA

Regions	Cultivated years	Type of root[a]	Diameter (mm)[b]	Part of root	Content (mg/100 g)															
					1	2	3	4	5	6	7	8	9	10	11	TIF[c]	AG IV	AG II	AG I	TAG[d]
ShanXi	10	TR	9.9	Xylem	44.3	43.4	46.8	30.7	1.4	15.9	43.5	26.6	40.0	5.4	9.6	307.7	1.9	3.9	6.0	11.8
				Bark	29.9	29.8	23.9	21.6	1.9	8.8	20.0	19.9	19.0	1.5	13.9	190.1	3.8	8.9	84.4	97.1
		BR	4.4	Xylem	49.4	37.9	66.9	44.1	5.7	7.1	48.5	34.6	18.6	2.1	10.0	324.9	3.4	3.2	6.9	13.5
				Bark	45.6	33.2	36.8	24.0	4.3	8.6	19.6	17.4	23.4	1.6	24.0	238.5	2.8	8.6	116.7	128.1
	9	TR	10.4	Xylem	26.6	39.3	20.0	14.0	3.3	10.8	31.5	22.0	26.6	5.1	18.4	217.7	3.5	3.1	5.1	11.6
				Bark	16.6	37.2	17.2	10.7	2.8	5.5	25.6	18.7	8.5	1.2	10.3	154.3	14.4	29.5	76.8	120.7
		BR	5.8	Xylem	27.6	31.3	24.7	8.4	2.2	5.7	31.5	11.7	5.0	0.7	9.0	157.9	1.7	1.2	2.7	5.6
				Bark	20.6	22.2	15.1	9.4	3.2	5.1	17.2	10.6	10.0	1.5	17.9	132.8	15.1	28.5	136.0	179.5
	8	TR	8.7	Xylem	22.7	36.6	15.9	10.5	1.6	10.2	25.2	17.0	44.8	2.2	3.7	190.3	5.0	6.0	12.1	23.1
				Bark	20.3	36.6	12.0	11.2	1.7	10.3	21.1	19.5	26.2	1.0	5.7	165.7	15.2	31.9	83.4	130.5
		BR	4.0	Xylem	25.1	33.1	19.2	12.2	2.9	7.9	29.8	16.7	34.0	3.0	6.2	190.2	4.6	3.8	8.6	17.0
				Bark	13.6	25.1	11.2	15.0	4.9	7.9	21.6	30.1	40.3	3.8	12.2	185.8	8.9	6.3	137.3	152.5
	7	TR	7.7	Xylem	28.5	35.7	23.8	18.2	5.2	11.8	19.3	25.2	36.0	4.2	14.3	222.0	4.6	4.4	6.6	15.7
				Bark	24.6	44.9	15.1	14.7	3.4	8.1	21.6	25.4	17.4	1.8	14.3	191.4	3.7	7.4	76.0	87.1
		BR	4.3	Xylem	23.9	43.0	29.2	14.6	4.7	8.7	33.9	29.0	23.1	2.2	26.7	239.1	5.7	3.6	6.1	15.4
				Bark	26.5	52.9	18.4	11.4	6.2	5.0	27.4	21.6	30.8	2.3	39.8	242.3	2.6	4.6	62.7	69.9
	6	TR	7.2	Xylem	20.4	33.3	16.1	24.2	5.4	13.1	25.4	30.2	24.8	3.6	15.9	212.4	3.1	2.8	2.8	8.7
				Bark	14.9	23.3	8.3	22.3	4.0	8.1	12.4	32.2	11.7	1.7	11.0	149.8	6.8	17.1	44.9	68.8
		BR	3.8	Xylem	17.3	20.3	12.2	24.5	6.9	10.3	13.7	28.9	17.8	2.6	27.1	181.4	3.8	2.1	3.4	9.3
				Bark	11.1	16.7	5.9	20.7	8.7	7.7	8.3	27.2	24.6	3.6	38.4	172.8	1.5	3.8	107.9	113.2
	5	TR	6.9	Xylem	21.2	19.6	13.4	24.2	0.7	20.6	12.4	23.8	31.9	2.3	5.8	176.0	2.8	3.7	6.2	12.7
				Bark	15.7	13.9	7.5	19.0	1.0	10.9	5.8	17.2	12.2	0.9	8.1	112.3	2.6	4.8	117.7	125.2
		BR	4.2	Xylem	21.8	13.8	19.5	23.4	7.9	10.3	12.2	15.7	17.1	1.6	17.7	160.9	6.4	3.4	3.9	13.7
				Bark	15.3	12.6	7.4	17.7	7.9	6.0	5.2	16.2	9.6	1.1	19.7	118.9	3.3	8.6	137.5	149.4
GanSu	3	TR	8.7	Xylem	28.0	12.7	14.6	9.1	53.1	9.2	33.5	8.4	41.2	11.3	22.6	243.8	9.6	15.1	32.3	57.0
				Bark	32.4	16.3	10.9	9.9	52.9	6.9	23.5	8.3	36.8	10.7	18.2	226.6	5.0	36.3	109.8	151.2
		BR	4.7	Xylem	27.5	13.9	14.1	10.7	36.5	7.9	34.3	6.1	20.9	5.3	11.6	188.7	10.6	6.9	6.7	24.2
				Bark	16.6	8.1	12.4	15.2	19.4	8.5	18.1	6.5	27.8	8.0	8.8	149.4	5.1	33.8	251.3	290.2
	2	MixedR	3.0	Xylem	25.8	16.0	21.4	16.3	18.3	10.4	15.8	14.1	38.0	3.9	7.9	188.0	7.4	8.6	9.3	25.3
				Bark	19.8	17.5	14.1	7.1	21.9	2.9	15.5	14.2	24.9	5.9	12.8	156.9	5.2	13.3	329.5	348.1
Inner Mongolia	3	TR	12.3	Xylem	66.8	51.5	68.5	34.6	46.9	5.9	49.6	16.3	41.8	9.8	11.5	403.3	5.7	3.9	22.0	31.5
				Bark	80.7	63.0	51.3	24.0	18.0	12.2	38.2	16.0	44.1	3.2	9.8	360.3	13.5	24.9	48.2	86.5
	3	TR	7.4	Xylem	91.8	38.7	69.8	40.1	52.6	5.6	32.3	14.6	47.5	4.3	4.2	401.5	4.9	3.7	18.2	26.8
				Bark	94.9	49.7	94.4	36.3	11.3	5.7	56.7	20.5	37.3	3.8	6.5	417.1	21.7	49.3	82.5	153.5
	3	TR	5.5	Xylem	53.3	57.2	42.8	16.5	50.0	4.6	37.9	16.2	25.2	2.3	4.1	310.3	2.7	4.3	21.9	29.0
				Bark	61.6	61.2	34.3	13.1	19.9	4.5	28.3	9.5	18.1	1.1	1.7	253.3	37.6	26.4	44.8	108.8
	3	TR	10.1	Xylem	57.9	45.2	34.0	31.0	7.3	33.7	25.4	23.3	89.0	9.3	94.1	450.3	15.9	7.0	17.4	40.3
				Bark	63.0	42.9	35.1	27.4	5.1	21.2	21.2	18.8	74.4	8.6	66.6	384.3	12.6	22.6	51.1	86.3
		BR	2.4	Xylem	48.4	29.0	25.1	29.7	7.7	24.0	16.6	20.5	104.1	10.8	61.2	377.1	9.2	14.0	23.2	46.4
				Bark	44.3	25.2	19.6	20.1	7.4	14.6	10.8	15.6	70.0	5.7	27.4	260.7	3.5	9.1	129.2	141.7
	2	MixedR	4.3	Xylem	23.1	28.7	33.6	14.0	9.8	12.9	43.5	14.4	125.2	10.5	53.8	369.4	8.4	3.8	8.7	20.8
				Bark	23.0	25.2	31.4	10.6	7.5	8.5	34.3	8.7	83.3	7.3	28.0	267.9	18.5	33.6	55.2	107.4
	3	TR	9.4	Xylem	55.9	26.1	42.3	29.0	23.8	6.3	10.6	5.5	109.3	8.0	19.8	336.5	4.9	4.7	6.6	16.2
				Bark	111.8	68.7	69.7	19.8	32.4	19.2	38.7	6.6	115.7	8.4	34.8	525.9	11.2	15.7	90.7	117.6
HeiLongJinang	3	BR	3.3	Xylem	99.7	58.1	102.6	36.5	63.6	22.5	80.5	14.8	107.4	14.9	68.0	668.5	2.5	9.3	25.6	37.4
				Bark	88.4	40.6	66.2	27.7	37.6	21.3	55.9	11.4	170.5	15.1	61.1	595.9	1.2	8.1	201.0	210.3
	3	TR	10.5	Xylem	62.6	33.7	35.8	45.9	13.4	59.2	19.7	22.5	133.2	9.2	76.7	511.9	6.6	34.4	27.6	68.6
				Bark	107.7	57.7	51.6	34.0	21.9	19.8	74.5	30.5	167.8	12.6	41.1	619.1	2.6	3.8	73.2	79.7

[a] TR and BR represent taproot and branch root, respectively. MixedR means that samples for taproot and branch root are not separated.

[b] The average xylem diameters are measured within same sample population.

[c] TIF represents average content of total isoflavonoids in the xylem or bark. No significance was found on TIF between xylem and bark ($P > 0.05$), on TIF in xylem between diameter smaller than 6 mm and larger than 6 mm ($P > 0.05$), and on TIF in bark between diameter smaller than 6 mm and larger than 6 mm ($P > 0.05$).

[d] TAG represents content of total astragalosides in the xylem and bark. Significant difference was observed on TAG between xylem and bark ($P < 0.001$). No significance was observed between in root with diameter of xylem smaller than 6 mm and larger than 6 mm ($P > 0.05$). Significant difference was observed between content of total astragalosides in the bark with diameter of xylem smaller than 6 mm and larger than 6 mm ($P < 0.05$).

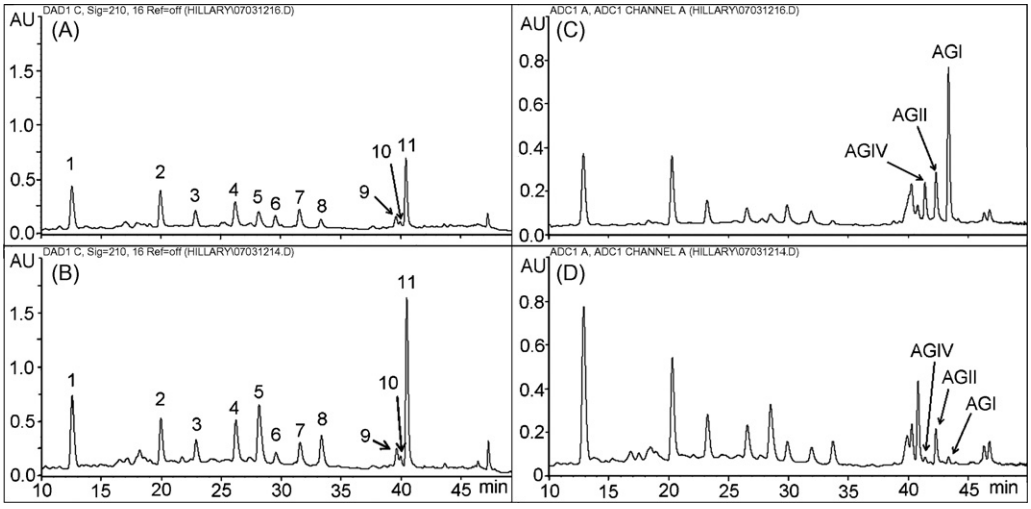

Fig. 1. Comparison of chromatographic profiles between xylem and bark of RA. (A) and (B) are extracts from bark and xylem of RA detected with UV at 210 nm; (C) and (D) are extracts from bark and xylem of RA detected by ELSD. Peaks were identified as: (**1**) calycosin-7-*O*-β-D-glucoside; (**2**) calycosin-7-*O*-β-D-glucoside-6′′-*O*-malonate; (**3**) ononin; (**4**) (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside; (**5**) (3*R*)-7,29-dihydroxy-3′,4′-dimethoxyisoflavan-7-*O*-β-D-glucoside; (**6**) calycosin; (**7**) formononetin-7-*O*-β-D-glucoside-6′′-*O*-malonate; (**8**) astrapterocarpanglucoside-6′′-*O*-malonate; (**9**) formononetin; (**10**) (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan; (**11**) (3*R*)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan; (**AG I**) astragaloside I; (**AG II**) astragaloside II and (**AG IV**) astragaloside IV.

The contents of eight compounds **1**, **3**, **4**, **6**, **9**, **AG I**, **AG II**, and **AG IV** were calculated using the respective calibration curves. The reference standards of compounds **2**, **5**, **7**, **8**, **10**, and **11** were not easily obtained, therefore their accurate contents could not be determined. Since compounds **2**, **7**, **8**, and **10** are derivates of compounds **1**, **3**, and **4**, respectively; while the chemical structures and UV spectrum of compounds **5** and **11** are similar to compound **4**, the contents of compounds **2**, **5**, **7**, **8**, **10**, and **11** were roughly estimated with similar calculation to the previous publication [8]. Contents of constituents in the xylem and bark of RA from each sample population are listed in Table 2.

AG I was the most abundant saponin in the xylem and bark. Contents of astragalosides in the bark were much higher than those in the xylem (Table 2). The highest ratio of total astragalosides between the bark and the xylem were up to 74-fold. The measured values of total astragalosides contents ranged from 1.6 to 78.6 mg/100 g with an average of 24.0 ± 16.1 (mean ± S.D., mg/100 g, $n = 70$), and 25.7–393.5 mg/100 g with an average of 138.5 ± 75.0 (mean ± S.D., mg/100 g, $n = 70$) for xylem and

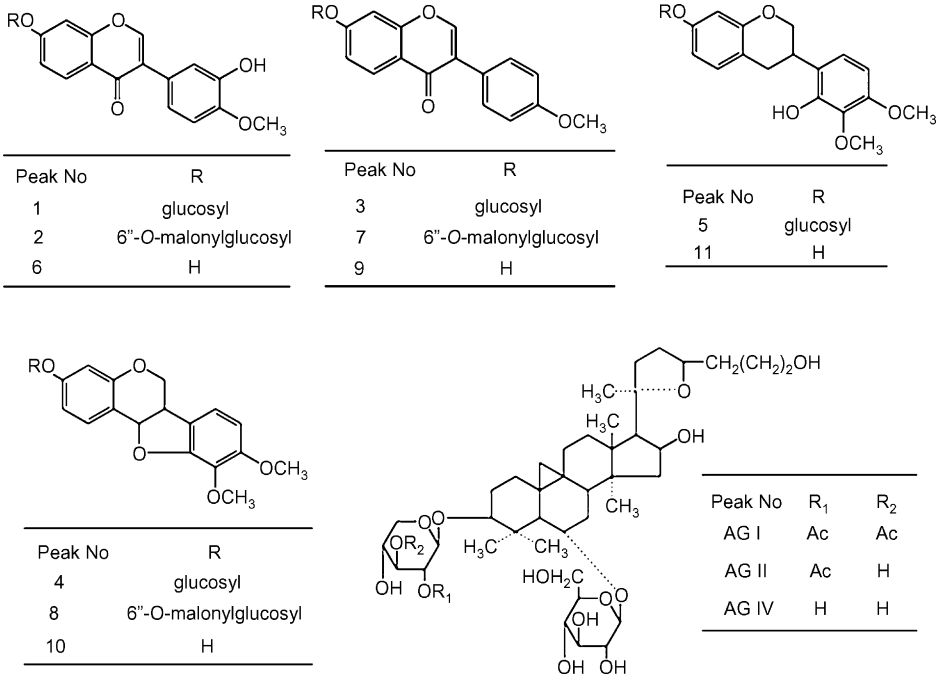

Fig. 2. Chemical structures of major isoflavonoids and astragalosides in RA.

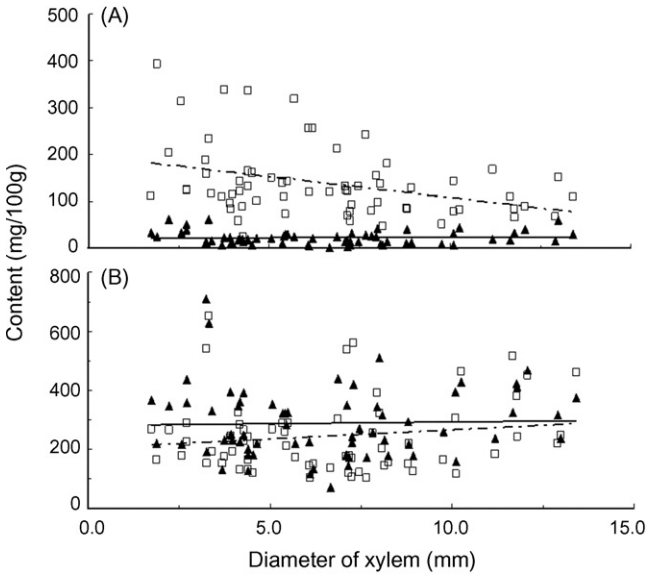

Fig. 3. Plots of diameter of xylem vs. content of total astragaloside (Panel A), total isoflavonoids (Panel B). In panel A, blank square (□) and solid triangle (▲) represents total astragalosides and total isoflavonoids in the bark, respectively; dash line (– · –) and solid line (—) represents trend line of total astragalosides and total isoflavonoids in the bark vs. diameter of xylem of RA, respectively. In panel B, blank square (□)) and solid triangle (▲) represents total astragalosides and total isoflavonoids in the xylem, respectively; dash line (– · –) and solid line (—) represents trend line of total astragalosides and total isoflavonoids in the xylem vs. diameter of xylem of RA, respectively.

bark, respectively. Despite the differences between the cultivated sites and cultivated years, contents of total astragalosides in the bark increased dramatically as the diameter of xylem decreased. Even in the same sample population, the amount of total astragalosides in the bark of branch root was higher than that in the taproot (Fig. 3A). This finding indicated that the contents of total astragalosides are roughly related to the diameter of the root, but not absolutely dependent on the cultivated years. These results indicated that it is not practicable to use size as quality assessment criteria.

It was shown that contents of total isoflavonoids ranged from 70.2 to 709.5 mg/100 g with an average of 291.7 ± 124.3 (mean ± S.D., mg/100 g, $n = 70$), and 100.2–653.4 mg/100 g with an average of 251.6 ± 136.7 (mean ± S.D., mg/100 g, $n = 70$) in the xylem and bark, respectively. Although the content of total isoflavonoids varied between samples, the average content of total isoflavonoids in the xylem was slightly higher than that in the bark, and contents of total isoflavonoids in the bark and xylem slightly increased as the diameter of xylem increased (Fig. 3B). Statistically, no significant differences were found, either on total qualified isoflavonoids between xylem and bark, or between thin roots (diameter of xylem <6 mm) and thick roots (diameter of xylem >6 mm, $P > 0.05$).

In addition, it was found that the chemical profile of constituents in the bark and xylem of RA from different cultivated sites showed a geographic region pattern. As illustrated in Table 2, compared to samples from Shanxi and Gansu, those from Inner Mongolia and Heilongjiang had a significantly higher level of isoflavonoids in both the bark and the xylem; samples from Gansu had significantly higher contents of astragalosides than those from other regions. This suggested that the differences between xylem and bark might be used as characteristics for distinguishing cultivated sites.

3.5. Principal component analysis

PCA data reduction allowed for the analysis of the main sources of variance present in the data set and the establishment of relationships between the objects (samples) and the variables (individual constituents).

Initially, data of all measured constituents in the xylem, bark and combined xylem with bark were analyzed with PCA to test clustering of cultivated regions. However, the results showed that it was unable to distinguish them. Since compounds **2**, **7**, and **8** are flavonoid glycoside malonates, they are very unstable and can be easily converted into their related glycosides **1**, **3**, and **4**, respectively during the extraction and analysis process [8]. The values of compounds **1**, **3**, and **4** might thus contain the degradation products of compounds **2**, **7**, and **8**, respectively. Similarly, **AG I** could also be decomposed to **AG II** and **AG IV** during extraction. These conversions led to increasing variance of the measured amounts of related compounds, making it impossible to accurately reflect the original information of the RA root and resulting in increased artificial error of PCA. Therefore, the measured constituents were divided into different groups based on their chemical structures in order to improved specificity of differentiation.

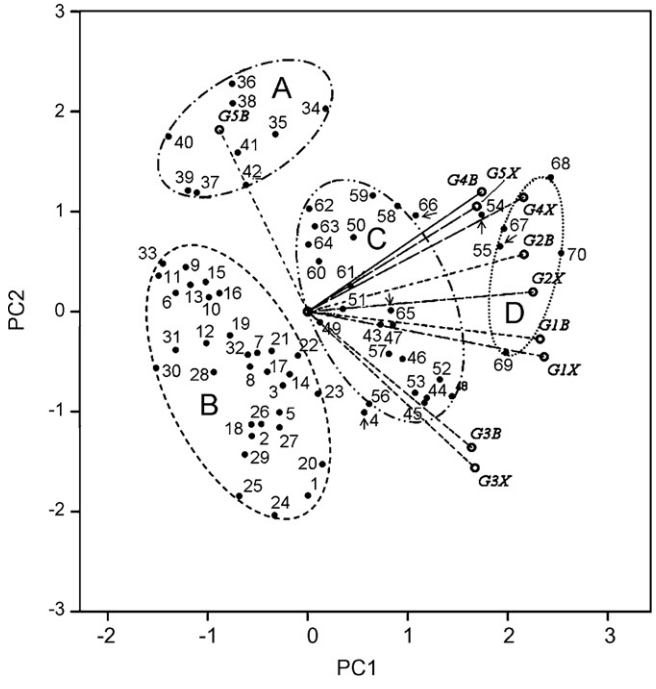

Fig. 4. Biplot of 70 RA samples and five categorized constituents in xylem and bark defined by the two principal components. **G1X–G5X** represented categorized constituents **G1–G5** in xylem; **G1B–G5B** represented categorized constituents **G1–G5** in bark. Data point #1–33 are samples from Shanxi (B); data point #34–42 are from Gansu (A); data point #43–64 are from Inner Mongolia (C) and data point 65–70 are from Heilongjiang (D). Arrows indicated outlier data (#4, #54, #55, #65, and #66).

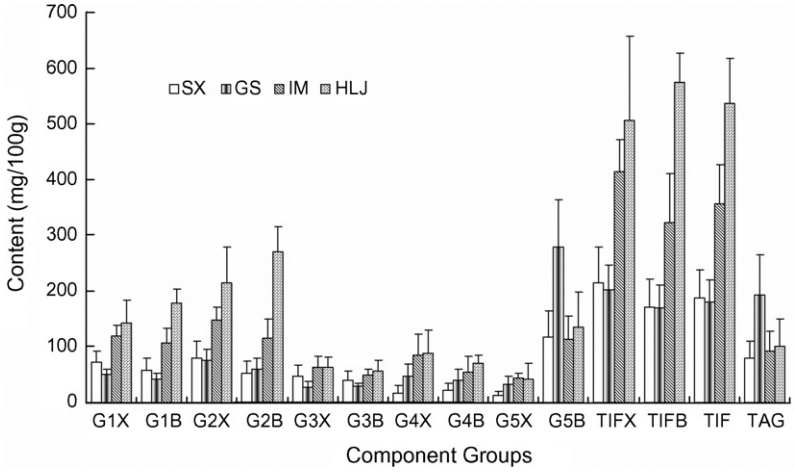

Fig. 5. Average contents of the categorized constituents from different cultivated sites. SX, GS, IM and HLJ represent Shanxi, Gansu, Inner Mongolia and Heilongjiang, respectively. **G1X–G5X** represent constituents of Group 1 to Group 5 in xylem; **G1B–G5**B represent constituents of Group 1 to Group 5 in bark. TIFX represents total isoflavonoids in xylem; TIFB represents total isoflavonoids in bark; TIF and TAG represent total isoflavonoids and total astragalosides in whole root of RA, respectively, which calculated by:

$$\mathrm{TIF} = \frac{\mathrm{TIFX} + \mathrm{TIFB} \times \mathrm{Fw}}{1 + \mathrm{Fw}};$$

$$\mathrm{TAG} = \frac{\mathbf{G5X} + \mathbf{G5B}(\mathrm{Fw})}{1 + \mathrm{Fw}};$$

where Fw = (weight of bark)/(weight of xylem) in the same root.

According to their chemical structures, the determined constituents could be categorized in five groups: (1) Group 1 (**G1**), contained calycosin and related constituents, which including compounds **1**, **2**, and **6**; (2) Group 2 (**G2**), contained ononin and related constituents which including compounds **3**, **7**, and **9**; (3) Group 3 (**G3**), contained (6a*R*,11a*R*)-3-hydroxy-9,10-dimethoxypterocarpan and related constituents which including compounds **4**, **8**, and **10**, (4) Group 4 (**G4**), contained (3*R*)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan and related constituents which including compounds **5** and **11**; and (5) Group 5 (**G5**), the astragalosides group which including **AG I**, **AG II**, and **AG IV**. In this study, constituents in the xylem and bark were determined. In addition, the integration of categorized constituents eliminated the artificial errors caused by the degradation during analysis, resulting in improved specificity of differentiation. When using the contents of the categorized constituents in the xylem and bark for the PCA analysis, three factors (principal components, PCs) were identified with initial eigenvalues >1, which explained 73.01% of the total variance of the data set. **PC 1** (explaining 48.3% of the variance) was mainly characterized by the categorized constituents **G1**, **G2**, **G4**, and **G5** in the xylem (which marked as **G1X**, **G2X**, **G4X**, and **G5X**, respectively), and categorized constituents **G1**, **G2**, and **G4** in the bark (which marked as **G1B**, **G2B**, and **G4B**, respectively), while **PC 2** (explaining 15.6% of the variance) was correlated with the categorized constituents **G3** in the xylem (marked as **G3X**), and **G3** and **G5** in the bark (marked as **G3B** and **G5B**, respectively). Fig. 4 presented the PCA score plot (**PC1** *vs.* **PC2**) resulting in four groups of RA to be differentiated. Although several outlier data were observed, the result illustrated that RA from Gansu, Shanxi, Inner Mongolia and Heilongjiang provinces could be well differentiated from each other.

3.6. Quality assessment

The quality of RA from four cultivated regions was assessed by the categorized constituents. The average contents of categorized constituents (**G1–G5**), total isoflavonoids and total astragalosides in the xylem and bark are summarized in Fig. 5. The categorized constituents **G1**, **G2**, and **G5** represented compounds related to calycosin, ononin and astragalosides. These components showed major aspects of biological activities of RA [24–33], suggesting that it is practical to use the categorized constituents for quality assessment. The highest contents of **G1**, **G2** and total isoflavonoids were found in samples from Heilongjiang. The content of **G5** (total astragalosides) was highest in samples from Gansu, about 2-fold higher than those from Shanxi, Heilongjiang and Inner Mongolia. In samples from Gansu Province, the total contents isoflavonoids and astragalosides were similar, whereas in samples from Shanxi, Inner Mongolia and Heilongjiang Provinces, contents of total isoflavonoids were much higher than that of total astragalosides.

In this study, it was found that astragalosides were concentrated in the bark of RA. Since the portion of bark in a thin root is much larger than that in a thick one, the overall contents of saponins in the thick RA were lower than in thin ones. A similar phenomenon was also found in other plants, such as ginseng [34]. Therefore, it is necessary to evaluate the reasonableness of traditional criteria for quality control of herbal materials of traditional Chinese Medicines.

4. Conclusion

In this paper, isoflavonoids and astragalosides in xylem and bark of RA from different cultivated regions in China were determined. The results showed that contents of astragalosides in the bark were much higher than in the xylem. Although contents of isoflavonoids varied between samples, no statistical significances on the content of isoflavonoids were found between the xylem and bark, or between thin and thick roots. According to their chemical structures, constituents in RA were categorized into five groups. Integrated content of the categorized constituents could eliminate the measurement error derived from relatively unstable compounds and could efficiently differentiate RA from different cultivated regions. Furthermore, the quality of RA from different cultivated regions was evaluated by comparing the integrated content of categorized constituents. It was shown that thin roots of RA contained more active constituents than thick roots, suggesting that the qualities of thin roots are better than thick roots.

Acknowledgment

This research is funded by Hong Kong Jockey Club Charities Trust.

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