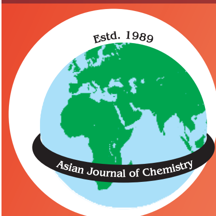

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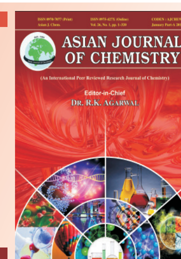

Activated-Constituents in the Rhizomes and Roots of *Rheum tanguticum*

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The rhizomes and roots of *Rheum tanguticum* are both used as rhubarb, a traditional Chinese medicine (TCM) in China. The activated-constituents, including free anthraquinones, anthraquinone glycosides, dianthrones, flavan-3-ols and hydrolyzable tannins were determined in the aforementioned two medicinal parts by RP-HPLC technique. The results showed the contents of the activated constituents in the roots were generally higher than those in rhizomes, meaning maybe that the medicinal quality of the roots of rhubarb is superior to the rhizomes. These observations do not support the traditional views.

Keywords: Rhubarb, *Rheum tanguticum*, Roots, Rhizomes, Activated constituents.

INTRODUCTION

Rhubarb is used in clinical contexts in Asia as a purgative, stomachic, astringent, tonic and antisporiatic drug[1-5]. It is also one of the oldest and most well-known Chinese herbal medicines (Flora Reipublicase Popularis Sinicase)[6]. A variety of bioactive ingredients, including free anthraquinones, anthraquinone glycosides, dianthrones, flavan-3-ols and hydrolysable tannins have been reported from rhubarb[7,8]. The chemical constituents of rhubarb have been shown to possess various bioactivities corresponding to its traditional use, in which free anthraquinones and liposoluble constituents have been shown to possess antiinflammatory and antibacterial effects[9-11]. The anthraquinone glycosides and dianthrones, both with strong polarity, exhibited to induce diarrhea[12,13]. In addition, the polyphenols, flavan-3-ols and gallic acid displayed to remove blood stasis and hemostasis[14]. In general, the different types of chemical constituents in rhubarb represent diverse biological activities. Here, we name those chemical constituents from rhubarb as the "activated constituents".

Rhubarb is the root and rhizome of several species being medical-part in the genus *Rheum.* According to the Pharmacopoeia of the People's Repbulic of China (2010 edition), the rhizomes and roots of *R. palmatum*, *R. tanguticum* and *R. officinale* are officially considered as rhubarb[6]. The rhizome is the stoutest part of the medicinal species of *Rheum* (Fig. 1A). The Japanese Pharmacopoeia (15th edition, 2006), specifies that rhubarb is only derived from the dried rhizomes of *R. palmatum*, *R. tanguticum*, *R. officinale*, *R. coreanum* NAKAI and their interspecific hybrids, but does not include the roots[15]. Several researches prompted that the medicinal quality of the rhizomes of rhubarb is higher than that of the roots[16-20]. In addition, comparison and analysis of the concentration of anthraquinones in the two parts of *Rheum*, aerial parts and underground parts, the former is higher than the latter[21]. In the traditional view, the quality of rhizomes is superior to that of roots[22]. But there is no evidence that the chemical constituents and related pharmacological activities of the roots and rhizomes of rhubarb are different. Therefore, as a part of our ongoing quality investigations of rhubarb, a detailed study on the active ingredients differences between *Rheum* rhizomes and roots was carried out.

The plant *R. tanguticum* not only is included in the Chinese and Japanese Pharmacopoeia, but also is important officinal varieties heavily cultivated in South China. And, the rhizomes and roots of *R. tanguticum* were generally used in folk. Thus, we selected this species to probe rhizomes and roots the same and different. The activated-constituents were classified into three types according to pharmacological activities: the free anthraquinones, anthraquinone glycosides and dianthrones and flavan-3-ols and gallic acid. These three groups were characterized according to HPLC. This paper presents the details of the study.

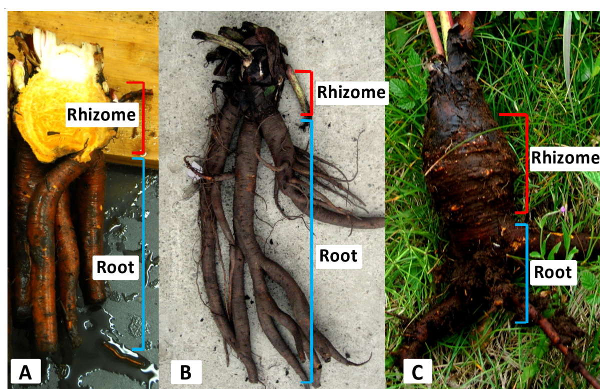

Fig. 1. Rhizome and root in wild and cultivated rhubarb (A) longitudinal section of rhizome. (B) cultivated rhubarb (strong root). (C) wild rhubarb (strong rhizome)

EXPERIMENTAL

Chemical and herbal materials: The standard compounds included five anthraquinones [rhein (**1**), emodin (**2**), aloe-emodin (**3**), chrysophanol (**4**) and physcion (**5**)], two dianthrone glycosides [sennoside A (**6**) and sennoside B (**7**)], one flavan-3-ol [catechin (**8**)] and one hydrolysable tannin [gallic acid (**9**)]. The standards were purchased from the National Institute for Food and Drug Control (Beijing, China) and Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). All standards had a purity of at least 98 % based on the HPLC profile, UV, MS, ^{1}H NMR and ^{13}C-NMR data. HPLC grade methanol, acetonitrile, tetrahydrofuran, phosphoric acid were purchased from Acros Organics (Fisher, USA). All remaining solvents used in the study were of analytical grade.

Twenty-four samples (sample code 1-24) of *R. tanguticum* were collected from Ruoergai in Sichuan Province in China. The growing time was March 2005 while harvesting time was June 2010 and the breeding methods is for seed production in all the 24 samples authenticated by one of the authors Dr. S.L. Wei (Beijing University of Chinese Medicine). Voucher specimens are stored at the School of Chinese Materia Medica, Beijing University of Chinese Medicine.

Samples preparation: Due to the distinct solubility of the reference chemicals, rhein, emodin, aloe-emodin, chrysophanol and physcion were dissolved in pure methanol, sennoside A and sennoside B were dissolved in 0.1 % $NaHCO_3$ solution and gallic acid and catechin were dissolved in 30 % methanol, as stock solutions, respectively. A defined amount of the above stock solutions were mixed and diluted to an appropriate concentration as standard stock solution. These standards were stable at least for two weeks at 4 °C.

The extracts of free anthraquinones, anthraquinone glycosides, dianthrones and flavan-3-ols and gallic acid from rhubarb were prepared according to published methods[3].

HPLC procedure: An Agilent HP 1100 series HPLC, with binary pump, DAD detector (Agilent Palo Alto, CA, USA), auto sampler and thermo-stated column compartment was used. The chromatographic separation was carried out using an Agilent Zorbax SB-C18 column (250 mm × 4.6 mm I.D. 5 µm) at a column temperature of 25 °C.

The free anthraquinones and anthraquinone glycosides were examined using a mobile phase of 0.1 % phosphoric acid in water (A) and methanol (B). The mobile phase was run using a gradient program as follows: 80 % (B) in 0-10 min, 80-85 % (B) in 10-15 min and 85 % (B) in 15-30 min. The flow rate was 1 mL min^{-1} and the DAD detector was set at 254 nm for acquiring chromatograms. This HPLC method is referred to as **HPLC procedure 1**.

Dianthrones were analyzed with the DAD detector set at 350 nm. The mobile phase consisted of chloromethane-water-glacial acetic acid (2:80:1.5, v/v/v) (A) and acetonitrile (B). Chromatographic separations were run at 0.8 mL min^{-1} flow rate, with a gradient of 15 to 20 % of solvent B in 0-30 min. This HPLC method is referred to as **HPLC procedure 2**.

Flavan-3-ols and gallic acid were separated using a mobile phase of 0.5 % glacial acetic acid in water (A) and methanol (B). The separation was done with a gradient program of 15 % (B) in 0-10 min and 15-25 % (B) in 10-40 min. The flow rate was 1 mL min^{-1} and the separation were monitored at 270 nm. This HPLC method is referred to **HPLC procedure 3**.

Validation procedure, precision, repeatability and accuracy: The standard solutions were diluted to appropriate concentrations and then triplicate injected for each concentration. Each calibration curve was constructed by plotting peak areas *versus* concentrations of the corresponding standard solutions (µg/mL).

Precision and repeatability of the different HPLC programs for each compound class were evaluated through the nine injections of the same sample solution and the same sample to inject three replicates according to the established method programs, respectively. The method to study accuracy was determined by application of the standard addition method (Table-1).

Data analysis of chromatogram: Statistical analyses were conducted using SPSS 20 (SPSS Inc., Chicago, IL, USA). Statistical significance of the comparison between chemical constituents of the roots and rhizomes was done using a paired sample T-test.

RESULTS AND DISCUSSION

Optimization of HPLC conditions: Different types of columns and mobile phase compositions were tested in order to determine the optimal chromatographic conditions[1,3,8,23]. It was found that better separation and peak shapes were achieved by the Agilent Zorbax SB-C18 column (250 mm × 4.6 mm I.D. 5 µm). For the mobile phase, methanol was used as the organic phase due to its better performance than acetonitrile for anthraquinones, the flavan-3-ol and gallic acid separations. The signal of DAD detection is selected at wavelength of 254, 350 and 270 nm for anthraquinones, dianthrones and flavan-3-ols, respectively, according to their maximum UV absorption values. Chromatographic separation of mixed standards and samples are shown in Figs. 2-4. Chromatographic peaks were identified by comparing retention times and UV absorption spectra with those acquired for standards analyzed under the same chromatographic conditions. Selected samples were also spiked with the standard compounds to confirm peak identity.

Validation of method: The seven point calibration curves for all nine compounds showed a linear correlation between concentration and peak area. Calibration data indicated the

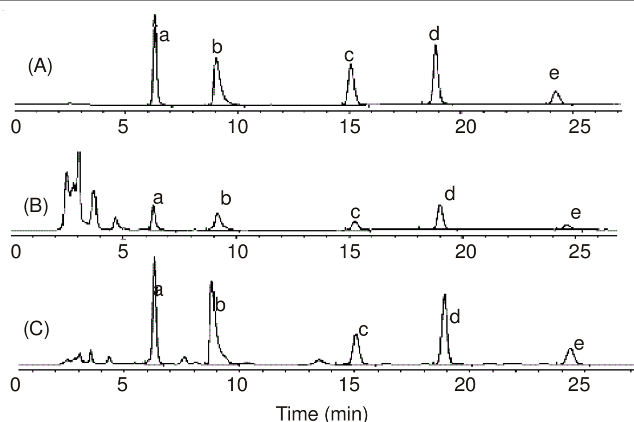

Fig. 2. (A) Chromatogram of mixed standards; (B) Chromatogram of free anthraquinones; (C) Chromatogram of anthraquinone glycosides. rhein (a), emodin (b), aloe -emodin (c), chrysophanol (d), physcion (e)

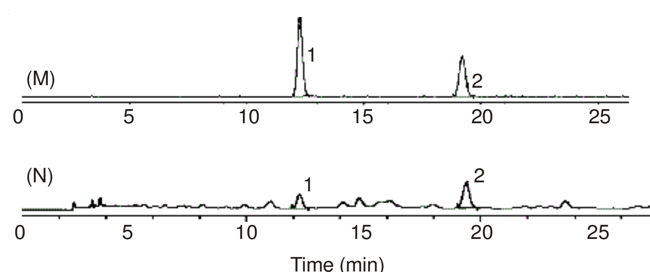

Fig. 3. (M) Chromatogram of mixed standards. (N) Chromatogram of dianthrones. Sennoside B (1), Sennoside A (2)

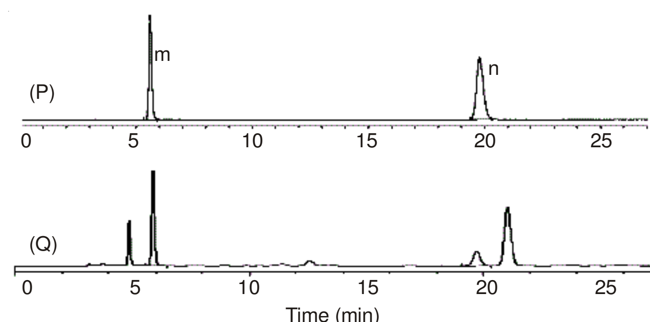

Fig. 4. (P) Chromatogram of mixed standards. (Q) Chromatogram of flavan-3-ols and gallic acid. gallic acid (m), flavan-3-ols (n)

linearity ($r^2 > 0.999$) of the detector response for all standard compounds, as shown in Table-1. All standards and samples were measured in triplicate. Multiple injections showed that the results are highly reproducible with low standard error. Accuracy of the method was confirmed by performing the aforementioned recovery experiment. The recoveries for all references were between 99.3 and 105 % and the RSDs were less than 3.9 % (Table-2). The above parameters demonstrated that the method is precise, sensitive and with high accuracy.

Free anthraquinones contents in rhizome and root of *R. tanguticum*: The free anthraquinones, tested here included rhein, emodin, aloe-emodin, chrysophanol and physcion (Table-3). Among the 24 tested samples of *R. tanguticum*, the free rhein content ranged from 0.03 to 0.65 % in the rhizomes, while the range was 0.04 to 1.17 % in the roots. The vast majority of rhizome samples had a rhein level concentrated from 0.10 to 0.30 %. These values were noticeably less than that of the roots, which had levels generally higher than 0.30 %. Only nine root samples had rhein less than 0.3 %. Emodin has been shown to be a potential agent that could reduce the impact of type II diabetes[5]. There was no marked difference in emodin content of rhizomes and roots in the samples examined in this study. However, emodin content was generally higher compared to that of rhein. Aloe-emodin content was generally similar to rhein content. However, aloe-emodin was not significantly different in roots compared to rhizomes, though appeared to have slightly higher concentration in the rhizome. Free chrysophanol, (abbreviated as f-Ch in Table-1) had the highest content of the free anthraquinones tested and was generally higher than 0.5 %. In fact, seven roots had chrysophanol levels higher than 1 % and one sample had a level of 3.31 %. The level of free physcion was the lowest of the five free anthraquinones studied. Most tested samples had physcion levels below 0.2 %, with eight samples below 0.1 %.

The aforementioned 24 samples as a whole, the five free anthraquinones content in the rhizome is lower than that of in the roots. To make an assay of each sample, the five free anthraquinones distributed was consistent in the rhizomes and roots of all samples tested, unified high or low. Studies have indicated that free anthraquinones in rhubarb contribute to antioxidant, antimicrobial and antiinflammatory activities of the plant[21,24,25]. Therefore, because the free anthroquinone levels are generally similar between roots and rhizomes, it can be preliminary concluded that it is likely the case that the antioxidant, antimicrobial and anti-inflammatory activities of the roots of *R. tanguticum* are the same as that of rhizomes.

In Table-4, the g-Rh (rhein glycosides), g-Em (emodin glycosides), g-AE (aloe-emodin glycosides), g-Ch (chrysophanol glycosides) and g-Ph (physcion glycosides) are the five free anthraquinones produced by acid hydrolysis. This method focuses on the aglycone without reference to location or type of glycoside. According to the results, anthraquinone glyco-

TABLE-1
CALIBRATION CURVES OF THE STANDARD COMPOUNDS **1-9**

HPLC conditions	Analyte	Calibration curve	r^2	Test range (μg/mL)
1	Rhein	Y = 5464.7 X-84.56	0.9995	0.014-2.7
1	Emodin	Y = 4435.0 X + 10.052	0.9998	0.11-3.3
1	Aloe-emodin	Y = 4943.4 X + 35.459	0.9996	0.015-5.4
1	Chrysophanol	Y = 5170.9 X-37.414	0.9994	0.014-5.4
1	Physcion	Y = 3167.5 X-12.006	0.9996	0.0061-3.0
2	Sennoside A	Y = 846.47 X-2.5209	1	0.023-1.6
2	Sennoside B	Y = 1059.5 X-0.777	1	0.029-1.9
3	Catechin	Y = 502.56 X + 0.6769	0.9999	0.013-3.8
3	Gallic acid	Y = 3300.8 X-30.586	0.9967	0.0013-0.57

TABLE-2
RECOVERIES OF THE NINE STANDARD CONSTITUENTS

Analyte	Contained (μg)	Added (μg)	Found (μg)	Recovery (%)	Mean (%)	RSD (%)
Aloe-emodin	0.079	0.07	0.15	98.6	100.2	2.19
	0.081	0.08	0.16	101.3		
	0.079	0.08	0.16	101.3		
	0.082	0.08	0.16	102.5		
	0.078	0.07	0.15	97.2		
Rhein	0.170	0.11	0.28	100.9	101.9	2.67
	0.168	0.13	0.30	98.5		
	0.171	0.13	0.29	100.8		
	0.167	0.14	0.30	105.3		
	0.165	0.13	0.30	104.0		
Rmodin	0.051	0.05	0.100	102.0	99.6	2.20
	0.051	0.05	0.103	98.0		
	0.053	0.05	0.102	98.0		
	0.053	0.05	0.104	98.0		
	0.052	0.05	0.101	102.0		
Chrysophanol	0.119	0.10	0.21	109.8	105.0	3.9
	0.124	0.11	0.23	103.7		
	0.121	0.11	0.23	100.9		
	0.122	0.11	0.23	101.8		
	0.119	0.11	0.22	108.9		
Physcion	0.051	0.05	0.100	102.0	99.3	2.67
	0.053	0.05	0.105	96.2		
	0.052	0.05	0.103	98.0		
	0.053	0.05	0.104	98.0		
	0.050	0.05	0.101	102.0		
Sennoside A	10.24	10.78	21.031	100.1	99.9	0.15
	10.27	10.05	20.289	99.7		
	10.25	10.05	20.288	99.9		
	10.25	10.09	20.347	100.0		
	10.24	10.10	20.335	99.9		
	10.23	10.19	20.426	100.1		
Sennoside B	3.472	3.49	6.990	103.3	101.8	1.61
	3.466	3.50	7.073	100.5		
	3.478	3.48	6.996	99.9		
	3.469	3.43	7.026	103.5		
	3.493	3.46	7.025	101.9		
Gallic acid	5.488	5.30	10.79	105.7	103.2	2.71
	5.410	5.91	11.31	104.2		
	5.434	5.80	11.23	104.7		
	5.120	5.62	10.72	98.6		
	5.318	5.80	11.12	102.4		
Catechin	21.10	26.6	47.70	99.6	100.2	2.42
	21.80	2.78	49.60	102.9		
	20.52	2.97	50.22	96.9		
	21.63	2.54	47.03	102.1		
	21.02	2.61	47.12	99.3		

sides are at higher concentrations than that of the aglycones. Aglycones are typically produced from emodin glycosides by acid hydrolysis. Glycosides are usually linked with carboxyl or hydroxyl groups or directly connected to the C-atom in benzene. Glycosides include a variety of saccharide units.

Aloe-emodin and physcion resulting from hydrolysis were about 1 to 3 % in the rhizomes and 2 to 4 % in the roots. Rhein, emodin and chrysophanol contents were generally higher than aloe-emodin and physcion and also tended to have higher concentrations in the roots.

The contents of sennoside A and B, flavan-3-ols and gallic acid in roots were generally higher than that of rhizomes in *R. tanguticum* (Tables 4-6).

Paired sample T-test was used to compare the differences between levels of chemical constituents found in the roots and rhizomes. In the T-test, $P < 0.05$ mean the different having statistical significance between the two control groups. $P < 0.01$, the difference between the compared groups is with high statistical significance. According to the T-test, the roots of *R. tanguticum* had higher contents in free rhein ($P < 0.05$), free emodin ($P < 0.05$) free chrysophanol ($P < 0.05$), free physcion ($P < 0.01$), rhein glycoside ($P < 0.01$), chrysophanol glycoside ($P < 0.01$), physcion glycoside ($P < 0.05$), sennoside B ($P < 0.01$), sennoside A ($P < 0.01$) and flavan-3-ols ($P < 0.01$) than that of the rhizomes (Table-7).

TABLE-3
CONTENTS (mg g-1) OF FIVE FREE ANTHRAQUINONES IN *R. tanguticum*

Sample Code	f-Rh		f-Em		f-AE		f-Ch		f-Ph	
	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root
1	0.06	0.17	0.28	0.44	0.06	0.11	0.19	0.24	0.09	0.11
2	0.15	0.38	0.28	0.42	0.10	0.19	0.35	0.63	0.19	0.31
3	0.14	0.34	0.23	0.42	0.19	0.31	0.50	0.82	0.20	0.33
4	0.28	0.28	0.12	0.11	0.19	0.21	0.83	1.04	0.28	0.30
5	0.14	1.17	0.19	1.17	0.14	0.75	0.66	3.31	0.16	0.48
6	0.12	0.12	0.19	0.17	0.25	0.16	0.45	0.46	0.21	0.16
7	0.04	0.12	0.18	0.39	0.06	0.14	0.17	0.33	0.06	0.10
8	0.65	0.64	0.42	0.41	0.77	0.42	0.91	0.90	0.28	0.24
9	0.46	0.90	0.69	1.66	0.51	0.70	0.98	1.51	0.40	0.54
10	0.12	0.16	0.25	0.31	0.20	0.20	0.32	0.34	0.09	0.11
11	0.05	0.14	0.14	0.20	0.09	0.12	0.17	0.42	0.08	0.13
12	0.13	0.04	0.24	0.18	0.09	0.05	0.23	0.21	0.08	0.07
13	0.24	0.25	0.40	0.44	0.28	0.23	0.48	0.42	0.16	0.15
14	0.13	0.35	0.26	0.54	0.07	0.15	0.21	0.41	0.09	0.14
15	0.36	0.71	0.29	0.49	0.27	0.41	0.46	0.82	0.27	0.43
16	0.27	0.19	0.40	0.38	0.33	0.26	0.57	0.48	0.31	0.27
17	0.20	0.24	0.29	0.36	0.19	0.18	0.47	0.42	0.16	0.16
18	0.43	0.51	0.44	0.49	0.30	0.27	0.90	1.44	0.25	0.37
19	0.34	0.31	0.25	0.24	0.28	0.22	0.61	0.61	0.20	0.20
20	0.39	0.39	0.40	0.19	0.29	0.27	0.53	1.01	0.38	0.31
21	0.34	0.79	0.19	0.45	0.28	0.51	0.60	1.15	0.19	0.37
22	0.09	0.53	0.24	0.77	0.07	0.24	0.28	1.19	0.07	0.26
23	0.13	0.20	0.16	0.24	0.08	0.11	0.20	0.30	0.09	0.15
24	0.08	0.12	0.18	0.17	0.08	0.08	0.28	0.35	0.09	0.08

TABLE-4
CONTENTS (mg g-1) OF ANTHRAQUINONE GLYCOSIDES AND SENNOSIDES IN *R. tanguticum*

Sample Code	g-Rh		g-Em		g-AE		g-Ch		g-Ph		SB		SA	
	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root	Rhizome	Root
1	5.12	3.20	7.80	6.31	2.70	1.96	4.54	2.93	2.24	1.82	3.56	8.23	5.98	16.22
2	4.62	7.03	3.99	5.49	1.65	2.11	3.71	5.13	2.06	2.95	1.31	2.78	3.86	9.07
3	4.62	5.40	4.28	4.80	3.51	3.54	5.31	5.69	2.02	2.43	2.43	4.71	7.07	13.45
4	5.02	5.77	1.69	1.39	2.92	3.85	7.95	8.88	3.24	3.18	0.68	1.25	16.7	3.91
5	5.20	4.15	3.92	3.61	2.60	1.83	10.84	9.45	2.34	1.89	0.85	2.36	2.99	6.64
6	4.17	5.08	4.17	4.87	4.24	4.49	6.14	7.78	3.31	3.03	2.16	3.69	6.09	10.65
7	3.10	3.90	6.32	6.84	2.75	3.25	4.73	5.06	1.60	1.64	3.22	5.62	7.78	13.62
8	4.30	6.35	1.93	3.34	4.58	3.28	4.71	7.08	1.91	2.72	3.61	2.24	9.32	5.75
9	2.18	2.94	1.52	4.10	1.46	1.93	4.27	5.26	2.19	2.58	2.42	5.15	22.56	29.51
10	3.82	3.51	5.38	4.15	4.04	3.10	6.26	4.75	2.06	1.64	3.14	4.38	9.59	20.66
11	3.30	5.30	3.60	4.88	2.68	2.58	5.94	9.71	2.77	3.28	0.76	1.87	2.82	5.82
12	2.45	2.46	2.13	1.49	1.38	0.97	4.59	4.78	1.73	1.30	1.91	2.71	6.03	8.97
13	3.31	3.26	4.57	3.09	2.40	2.36	4.37	4.06	1.98	1.82	3.43	4.7	19.43	21.53
14	4.09	4.33	4.52	2.60	1.43	1.36	3.60	3.91	2.07	2.05	1.64	4.89	5.11	16.11
15	4.45	8.50	3.27	4.23	2.98	4.50	3.15	6.89	2.71	4.51	3.37	7.72	13.42	24.00
16	2.96	5.55	4.57	5.76	3.29	5.43	4.87	7.92	3.47	5.24	6.70	7.51	17.65	21.78
17	3.42	3.18	2.70	3.06	2.08	1.93	5.09	4.46	2.27	2.08	1.93	2.76	10.57	15.35
18	5.02	6.15	3.61	4.41	2.73	2.84	9.29	10.43	3.41	3.23	2.26	3.47	10.97	10.24
19	4.00	6.22	2.37	3.47	2.03	2.66	4.74	6.47	1.76	2.38	1.39	2.10	10.76	12.58
20	3.25	6.07	1.34	2.99	2.97	3.84	6.11	8.27	2.06	3.25	0.87	3.19	11.44	9.83
21	5.58	6.08	2.72	2.95	3.67	3.63	4.59	5.16	2.35	2.47	6.09	7.42	13.91	18.63
22	3.45	10.02	2.31	12.04	1.61	3.44	6.59	14.86	1.64	3.92	1.69	5.03	4.75	15.92
23	4.68	7.30	4.04	5.00	1.65	2.58	3.49	5.82	2.27	3.35	4.14	5.49	12.34	15.25
24	2.59	5.12	3.00	4.66	2.05	2.46	4.95	8.31	1.80	2.11	2.04	2.83	6.56	7.79

g-Rh, Rhein glycoside; g-Em, Emodin glycoside; g-AE, Aloe-emodin glycoside; g-Ch, Chrysophanol glycoside; g-Ph, Physcion glycoside; SB, Sennoside B; SA, Sennoside A

TABLE-5
CONTENTS (mg g-1) OF GALLIC ACID AND FLAVAN-3-OLS IN *R. tanguticum*

Sample Code	GA		F3O		Sample Code	GA		F3O	
	Rhizome	Root	Rhizome	Root		Rhizome	Root	Rhizome	Root
1	1.29	1.15	24.51	23.47	13	1.26	1.26	17.70	23.72
2	1.56	1.63	18.39	17.52	14	0.85	0.89	18.82	21.39
3	1.00	1.24	19.26	20.31	15	1.14	1.24	20.94	26.78
4	0.97	1.32	16.60	17.02	16	0.96	0.82	15.46	24.15
5	1.71	5.19	22.06	21.18	17	1.06	1.38	13.66	13.94
6	3.06	2.36	10.94	16.45	18	0.62	0.59	18.99	23.09
7	0.76	0.87	19.80	18.64	19	1.24	1.47	15.48	19.76
8	1.55	2.13	11.79	16.16	20	0.97	0.85	21.21	27.27
9	3.71	8.69	23.08	28.62	21	1.60	1.77	18.52	20.38
10	1.26	1.19	19.41	20.55	22	0.55	4.65	24.1	23.78
11	1.36	2.32	17.66	22.89	23	0.91	0.83	20.15	27.16
12	1.14	0.90	16.75	21.6	24	0.75	0.50	22.86	22.63

GA, Gallic acid; F3O, Flavan-3-ol

TABLE-6
MEAN CONTENTS (mg g-1) OF FOURTEEN COMPOUNDS IN *R. tanguticum*

	No.	f-Rh	f-Em	f-AE	f-Ch	f-Ph	g-Rh	g-Em	g-AE	g-Ch	g-Ph	SB	SA	GA	F3O
T-rh	24	0.23± 0.15	0.29± 0.13	0.22± 0.16	0.48± 0.25	0.18± 0.10	3.95± 0.95	3.57± 1.55	2.64± 0.92	5.41± 1.82	2.30± 0.56	2.57± 1.54	9.90± 5.31	1.30± 0.72	18.67± 3.56
T-r	24	0.37± 0.29	0.44± 0.34	0.26± 0.18	0.78± 0.66	0.24± 0.13	5.29± 1.84	4.40± 2.12	2.91± 1.07	6.79± 2.66	2.70± 0.95	4.26± 2.00	13.89± 6.47	1.89± 1.85	21.60± 3.8

T-rh, rhizome of *R. tanguticum*; T-r, root of *R. tanguticum*; f-Rh, free rhein; f-Em,free emodin; f-AE, free aloe-emodin; f-Ch, free chrysophanol; f-Ph, free physcion; g-Rh, rhein glycoside; g-Em, emodin glycoside; g-AE, aloe-emodin glycoside; g-Ch, chrysophanol glycoside; g-Ph, physcion glycoside; SB, Sennoside B; SA, Sennoside A; GA, gallic acid; F3O, flavan-3-ols; No., number of the samples.

TABLE-7
COMPARISON OF A VARIETY OF REFERENCE COMPOUNDS IN *R. tanguticum*

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
f-Rh: rh-r	-0.145	0.252	0.052	-0.252	-0.039	-2.823	23	0.010*
f-Em: rh-r	-0.150	0.297	0.061	-0.276	-0.025	-2.485	23	0.021*
f-AE: rh-r	-0.043	0.168	0.034	-0.114	0.029	-1.238	23	0.228
f-Ch: rh-r	-0.307	0.560	0.114	-0.543	-0.070	-2.683	23	0.013*
f-Ph: rh-r	-0.056	0.095	0.019	-0.096	-0.016	-2.897	23	0.008**
g-Rh: rh-r	-1.340	1.782	0.364	-2.093	-0.588	-3.684	23	0.001**
g-Em: rh-r	-0.824	2.216	0.452	-1.760	0.111	-1.822	23	0.081
g-AE: rh-r	-0.272	0.839	0.171	-0.626	0.083	-1.586	23	0.126
g-Ch: rh-r	-1.385	2.144	0.438	-2.290	-0.479	-3.164	23	0.004**
g-Ph: rh-r	-0.400	0.773	0.158	-0.727	-0.074	-2.537	23	0.018*
SB: rh-r	-1.692	1.304	0.266	-2.242	-1.141	-6.356	23	0.000**
SA: rh-r	-3.975	5.388	1.100	-6.250	-1.700	-3.614	23	0.001**
GA: rh-r	-0.583	1.446	0.295	-1.194	0.027	-1.976	23	0.060
F3O: rh-r	-2.929	3.023	0.617	-4.206	-1.652	-4.746	23	0.000**

Conclusion

During the survey of *Rheum* samples for this study, wild-collected samples typically had longer and more developed rhizomes than cultivated samples (Fig. 1C). Cultivated samples of *Rheum* appear to have more developed roots but shorter rhizomes. Now wild populations of *Rheum* have been declining due to over-collection and *Rheum* cultivation has increased significantly. It is likely that roots of rhubarb may become the main source of medicinal materials in the future. Similarity the same for the *R. tanguticum*. In order to appraise the character, we contrasted with the above-mentioned parts. According to our study, the content of activated constituents in roots is generally higher than that in rhizomes.

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REFERENCES

1. W. Jin and P.F. Tu, *J. Chromatogr. A*, **1092**, 241 (2005).
2. W.Y. Li, S.W. Chan, D.J. Guo, M.K. Chung, T.Y. Leung and H.F.P. Yu, *J. Ethnopharmacol*., **124**, 251 (2009).
3. J. Liu, S.L. Wei, C.S. Liu and Y. Zhang, *Chin. J. Exp. Tradit. Med. Form*., **18**, 346 (2012).
4. H.R. Xiong, J. Luo, W. Hou, H. Xiao and Z.Q. Yang, *J. Ethnopharmacol*., **133**, 718 (2011).
5. J.F. Xue, W.J. Ding and Y. Liu, *Fitoterapia*, **81**, 173 (2010).
6. China Pharmacopoeia Committee, The Pharmacopoeia of People's Republic of China (I), Chemical Industry Press: Beijing, China, pp. 22-23 (2010).
7. J. Jin, H. Bi, J. Hu, H. Zeng, G. Zhong, L. Zhao, Z. Huang and M. Huang, *Phytother. Res*., **25**, 1250 (2011).
8. G. Li and Z. Hu, *Analyst*, **123**, 1501 (1998).
9. Y.N. Cai and Q.H. Chen, *J. Chin. Pharm. Univ.*, **19**, 282 (1988).
10. S.K. Agarwal, S.S. Singh, S. Verma and S. Kumar, *J. Ethnopharmacol*., **72**, 43 (2000).
11. Y. Xu, S. Cao and G.T. Qu, *Chinese J. Information TCM*, **14**, 43 (2007).
12. P. Xiao, L. He and L. Wang, *J. Ethnopharmacol*., **10**, 275 (1984).
13. Y. Qin, J.B. Wang, W.J. Kong, Y.L. Zhao, H.Y. Yang, C.M. Dai, F. Fang, L. Zhang, B.C. Li, C. Jin and X.H. Xiao, *J. Ethnopharmacol*., **133**, 1096 (2011).
14. T. Kosuge and H. Ishida, *Chem. Pharm. Bull. (Tokyo)*, **33**, 1503 (1985).
15. Japanese Pharmacopoeia Committee, The Japanese Pharmacopoeia; The Society of Japanese Pharmacopoeia: Tokyo, Japan, edn 15 (2006).
16. H.Y. Xiong, X.F. Zhang, H. Wang and L. Pan, *Sin*, **23**, 328 (2003).
17. Z. Kerem, I. Bilkis, M.A. Flaishman and L. Sivan, *J. Agric. Food Chem*., **54**, 1243 (2006).
18. B.A. Zargar, M.H. Masoodi, B. Ahmed and S.A. Ganie, *Food Chem*., **128**, 585 (2011).
19. M.Y. Yoo, K.S. Oh, J.W. Lee, H.W. Seo, G.H. Yon, D.Y. Kwon, Y.S. Kim, S.Y. Ryu and B.H. Lee, *Phytother. Res*., **21**, 186 (2007).
20. M. Dregus and K.H. Engel, *J. Agric. Food Chem*., **51**, 6530 (2003).
21. J.H. Zheng and D.A. Guo, Modern Research on Rhubarb, Peking University Medical Press: Beijing, China, pp. 453-454 (2007).
22. A. Li, J.K. Wang and X.L. Meng, *China Pharmacy Publishing House*, **21**, 4036 (2010).
23. W. Jin, R.-L. Ge, Q.-J. Wei, T.-Y. Bao, H.-M. Shi and P.-F. Tu, *J. Chromatogr. A*, **1132**, 320 (2006).
24. X.Y. Cui, Y.L. Wang, N. Kokudo, D. Fang and W. Tang, *Trends*, **4**, 39 (2010).
25. Y.Q. Zhao, X.H. Liu, T. Ito and J.M. Qian, *World J. Gastroenterol*., **10**, 1005 (2004).