Comparative Study of Chemical Constituents of Rhubarb from Different Origins

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A comparative study of the pharmacologically active constituents of 24 rhubarb samples, which were identified genetically as *Rheum tanguticum*, 3 intraspecies groups of *R. palmatum* and *R. officinale*, was conducted using reversed-phase high performance liquid chromatography (HPLC) methods. Thirty compounds belonging to anthraquinones, anthraquinone glucosides, dianthrones, phenylbutanones, stilbenes, flavan-3-ols, procyanidins, galloylglucoses, acylglucoses, gallic acid, and polymeric procyanidins were analyzed quantitatively. The drug samples derived from the same botanical source showed similar chromatographic profiles, and the comparable specific shape that appeared in the 10-directed radar graphs constructed on the basis of the results of quantitative analysis indicated the relationship between chemical constituent patterns and genetic varieties of rhubarb samples.

Key words Rhei Rhizoma; *Rheum*; genetic variety; HPLC; quantitative comparison

Rhei Rhizoma (rhubarb), called Dahuang in Chinese, is widely known as a purgative and anti-inflammatory agent. In Chinese Pharmacopoeia,[1] the official Dahuang is prescribed as the dried rhizome and root of *Rheum palmatum* L., *R. tanguticum* MAXIM. ex BALF., and *R. officinale* BAILLON of the family Polygonaceae. On the other hand, the Japanese Pharmacopoeia[2] prescribes not only the above three species but also *R. coreanum* NAKAI, and their interspecific hybrids as the botanical origin for Rhei Rhizoma. *Rheum* plants are self-incompatible in nature, therefore random hybridization results in morphological intermediate forms in the shape of leaf blade, type of inflorescence, color of flowers, *etc.* This situation makes the taxonomic identification of *Rheum* plants very difficult. In recent years, quality degradation of Rhei Rhizoma that affects therapeutic efficacy was pointed out by Japanese Kampo doctors. As factors affecting the difference of chemical constituent composition and contents, many reasons such as genetic distinction, botanical sources, production areas, harvest time, processing method, *etc.*, are considered. Kashiwada *et al.*[3] reported the characteristic constituent pattern in Rhei Rhizoma produced in Qinghai and Sichuan Provinces; however, their botanical sources were not demonstrated due to the lack of suitable identification methods. In our previous paper,[4] chloroplast *mat*K gene sequence of *Rheum* plants was found a useful index to differentiate species and to deduce the production areas. Subsequently, by comparing nucleotide sequences of Rhei Rhizoma with those of *Rheum* species, the botanical origins of 22 rhubarb samples were determined.[4—6] On the other hand, for quantitative control of Rhei Rhizoma, HPLC methods to analyze 30 bioactive constituents have been developed.[7] In the present paper, quantitative analysis of 30 constituents of a total of 24 rhubarb samples obtained from Qinghai, Sichuan, Yunnan, and Gansu Provs. of China and from Japan was further performed to clarify the quality of marketing samples. Previously, consistency between the similarity of chemical constituent pattern and genetically close relatives of various taxa has been observed within the genera *Panax*,[8] *Glycyrrhiza*,[9] and *Psilocybe*.[9] Herein, we also investigate the relationship between chemical constituent patterns and genetic variation.

Experimental

Materials Eighteen crude drug samples were purchased in Chinese markets near the production area of *Rheum* species; five samples in Japan and one sample in Hong Kong (Table 1). Of these total 24 samples, 17 were provided for sequence analysis of the partial or entire *mat*K gene region as described in our previous papers.[4,6] Seven samples were newly analyzed by the modified molecular method, and chemical analysis of all 24 samples was carried out. Crude drug samples were deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW).

Molecular Identification Each one specimen from drug samples Nos. 3, 4, 7, 11, 18, 23, and 24 was analyzed as follows. Total DNA was extracted from 50—100 mg of rhizome powder using DNeasy™ Plant Mini Kit (QIAGEN, Germany). Two fragments (I, II) (Fig. 1) of 748 and 539 bp with partial overlap region in the partial *mat*K gene (1265 bp) were amplified *via* PCR method using 10—100 ng of total DNA as template (Fig. 1). The 50 μl of reaction mixture consisted of 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 μM of each primer, and 1.5 U *Taq* polymerase (Promega, U.S.A.). Two pairs of primers flanking the two different regions (I, II) of *mat*K gene were as follows: matKAF (5′-CTA TAT CCA CTT ATC TTT CAG GAG T-3′) and trnK1544R (5′-GGA TAA CCC CAG AAT GCT TAG-3′) for region I; matK780F (5′-ACT AAG CAT TCT GGG GTT ATC-3′) and matK8R (5′-AAA GTT CTA GCA CAA GAA AGT CGA-3′) for region II. PCR amplification was carried out in a Thermal Controller PTC-100 (MJ Research Inc., U.S.A.) with the following cycling condition: hot start at 94 °C for 5 min followed by 38 cycles of 94 °C for 1 min, 48 °C for 1 min, and 72 °C for 1.5 min, with final extension at 72 °C for 10 min. The 1/10 volume of the resulting PCR product was detected by 1.0% agarose gel electrophoresis then the remaining part was purified using a QIAquick PCR Purification Kit (QIAGEN, Germany). Sequencing reactions of the purified PCR products were carried out by BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, U.S.A.). Each sequence was determined directly by ABI PRISM 3100-*Avant* Genetic Analyzer (Applied Biosystems, U.S.A.) and analyzed by Sequencing Analysis Software (Version 5.2, Applied Biosystems, U.S.A.). The DNA sequences obtained were assembled and consensus sequences constructed by AutoAssemble program (Version 1.3.0, Applied Biosystems, U.S.A.).

Standard Samples and Reagents Anthraquinones (**1**—**5**; shown in Fig. 2), anthraquinone glucosides (**6**—**10**), dianthrones (**11**, **12**), phenylbutanones (**13**, **14**), stilbenes (**15**, **16**), flavan-3-ols (**17**, **18**), procyanidins (**19**, **20**), galloylglucoses (**21**—**24**), acylglucoses (**25**—**27**), gallic acid (**28**), RG-

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Table 1. Rhei Rhizoma Used in This Study and Their Botanical Sources

Sample No.	Herbal drug name	Market	Production area	Date of collection	TMPW No.[a)]	Botanic origin (type)[b)]	Sequence identity (Plant specimen Code No.)[c)]	GenBank accession No.
1	Dahuang (大黄)	Banma (班瑪) County, Qinghai (青海), China	Banma County, Qinghai	2000. 7	20061	*Rheum palmatum* (I)	Pq1	AB115669
2	Dahuang	Tongren (同仁) County, Qinghai, China	Huangnan (黄南) County, Qinghai	2000. 7	20065	*R. tanguticum*	T2	AB115682
3	*Dahuang	Tongren County, Qinghai, China	Huangnan County, Qinghai	2000. 7	20066	*R. tanguticum*	T2	AB115682
4	*Dahuang	Dulan (都蘭) County, Qinghai, China	Xiariha (夏日哈), Dulan County, Qinghai	2000. 8	20107	*R. tanguticum*	T4	AB115683
5	Dahuang	Dulan County, Qinghai, China	Reshui (熱水), Dulan County, Qinghai	2000. 8	20108	*R. tanguticum*	T2	AB115682
6	Dahuang	Dulan County, Qinghai, China	Balong (巴隆), Dulan County, Qinghai	2000. 8	20109	*R. tanguticum*	T4	AB115683
7	*Dahuang	Ganzi (甘孜) County, Sichuan (四川), China	Ganzi County, Sichuan	2000. 8	20218	*R. palmatum* (I)	(similar to Pq1)	
8	Dahuang	Kangding (康定) County, Sichuan, China	Jiulong (九龍) County, Sichuan	2000. 8	20216	*R. palmatum* (III)	Ps9	AB115678
9	Yin-huang (陰黄)	Yaan (雅安) County, Sichuan, China	Litang (理塘) County, Sichuan	2001. 2	20574	*R. palmatum* (III)	Ps14	AB115680
10	Yin-kang-huang (陰康黄)	Yaan County, Sichuan, China	Shimian (石棉) County, Sichuan	2001. 2	20573	*R. palmatum* (III)	Ps7	AB115677
11	*Yin-huang (陰黄)	Yaan County, Sichuan, China	Shimian County, Sichuan	2001. 2	20576	*R. palmatum* (II)	Pg25	AB115673
12	Dahuang	Wanyuan (万源) County, Sichuan, China	Wanyuan County, Sichuan	2000. 8	20266	*R. palmatum* (II)	Ps4	AB115672
13	Dahuang	Wanyuan County, Sichuan, China	Wanyuan County, Sichuan	2000. 8	20267	*R. officinale*	O4	AB115685
14	Mati-dahuang (馬蹄大黄)	Wanyuan County, Sichuan, China	Wanyuan County, Sichuan	1987. 10	07422	*R. palmatum* (II)	Ps4	AB115672
15	Dahuang	Zhongdian (中甸) County, Yunnan (雲南), China	Zhongdian County, Yunnan	1999. 7	19535	*R. palmatum* (II)	Pg25	AB115673
16	Ba-cheng-ji (八成吉)	Li (礼) County, Gansu (甘粛), China	Li County, Gansu	2001. 7	20926	*R. palmatum* (II)	Pg25	AB115673
17	Tong-huo (統貨)	Li County, Gansu, China	Li County, Gansu	2001. 7	20928	*R. palmatum* (II)	Pg25	AB115673
18	*Dahuang	Zhouqu (舟曲) County, Gansu, China	Zhouqu County, Gansu	2001. 8	20932	*R. palmatum* (III)	(similar to Ps12)	
19	Dahuang	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	Shimian County, Sichuan	2000. 4	19928	*R. palmatum* (III)	Ps7	AB115677
20	Liu-cheng-ji (六成吉)	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	Li County, Gansu	2000. 3	19927	*R. palmatum* (II)	Pg25	AB115673
21	Ya-huang (雅黄)	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	Sichuan	2000. 5	19948	*R. palmatum* (I)	Ps15	AB115671
22	Bao-huang (包黄)	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	Qinghai	2000. 5	19949	*R. tanguticum*	T2	AB115682
23	*Xiang-huang (箱黄)	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	Qinghai	2000. 4	19929	*R. palmatum* (I)	Pq3	AB115670
24	*Chang-tiao-jihuang (長条吉黄)	Tyou Koug You, Hongkong	Unknown	1980. 1	414	*R. tanguticum*	(similar to T2)	

* Newly provided samples for sequence analysis in this study. Their partial *mat*K gene sequences are shown in Fig. 1. *a*) The specimen reference number of the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama (TMPW). *b*) Type of *R. palmatum* is determined on the basis of the phylogenetic tree of *mat*K gene sequence using UPGMA method. *c*) Partial *mat*K gene sequences of the samples were identical to those of plant specimens with following code numbers. Locality of voucher, voucher number, and collection date are shown in ref. 4.

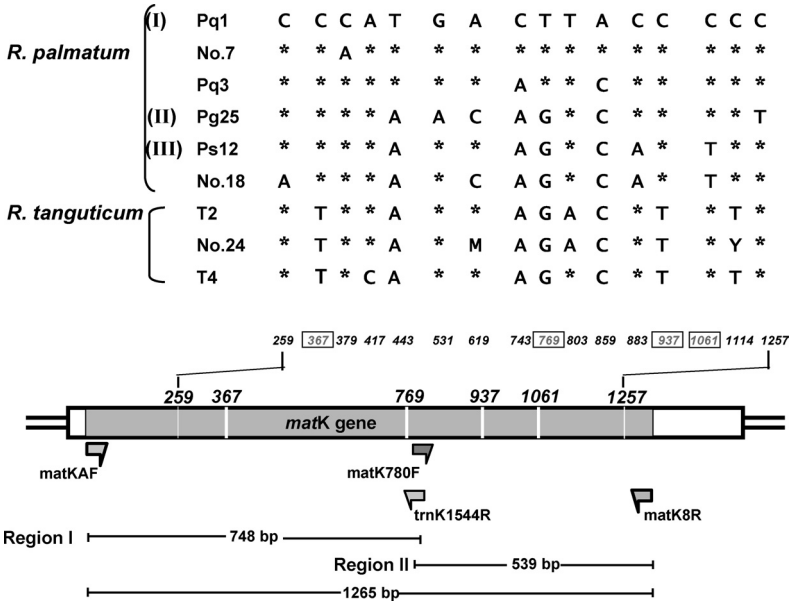


Fig. 1. Partial *mat*K Gene Sequences Observed in 7 Rhubarb Samples

Asterisks (*) indicate sequence identity with *R. palmatum* from Banma County, Qinghai Prov., China. M=A/C, Y=C/T. Pq1, Pq3, Pg25, Ps12, T2, and T4 are code numbers of plant specimens whose sequences were registered in GenBank and Nos. 7, 18, and 24 are rhubarb samples. The numerals in italics under the sequence indicate the aligned nucleotide position from the 5′ end of the *mat*K gene. The positions of marker nucleotides useful for classification are enclosed by boxes. Two pairs of primers, matKAF and trnK1544R flanking region I and matK780F and matK8R flanking region II, were used for PCR amplification. The *mat*K gene is 1518 bp in length.

tannin (**29**), and rhatannin (**30**) were isolated from the drug sample (No. 23); their structures are shown in Fig. 2. The isolated compounds were identified by comparison of their NMR and mass spectral data with those reported in the literature.[11—24] All chemicals were of analytical grade and chromatographic solvents HPLC grade.

Instrumentation and Analytical Conditions of HPLC Method The JASCO HPLC system (Jasco Co., Ltd., Japan) comprises a PU-1580 intelligent pump, DG-1580-53 3 line-degasser, LG-1580-02 ternary gradient unit, CO-1565 intelligent column oven, AS-2057 plus intelligent sampler, and MD-1510 diode array detector. An Inertsil ODS column (5 μm particle size, 4.6 mm i.d.×250 mm, GL Science Inc.) was used for all chromatographic experiments. The column temperature was set at 45 °C and eluted compounds were detected by monitoring the UV absorbance at 280 nm. The chromatographic data were collected and processed by Borwin-PDA Application and Borwin Chromatography Software (Version 1.5, Jasco Co., Ltd., Japan).

The mobile phase systems used in this study were the same as those described in our previous paper[7] (see Table 2).

Preparation of Standard Solution and Samples Stock solutions of each standard compound were independently prepared by dissolving the appropriate amount of the compound in methanol so as to obtain a final concentration of 1 mg/ml. To draw calibration curves a series of standard solutions were prepared from the stock solution then filtrated through a 0.2-μm Millipore filter (Advantec, Japan). Typical calibration curves containing 1, 5, and 10 μg of analytes were prepared plotting area against injection amount.

For quantitation of the constituents in rhubarb samples, 3 specimens were randomly selected from each sample and the central parts extracted and pulverized. The results of quantitation were presented as the average values of the 3 specimens.

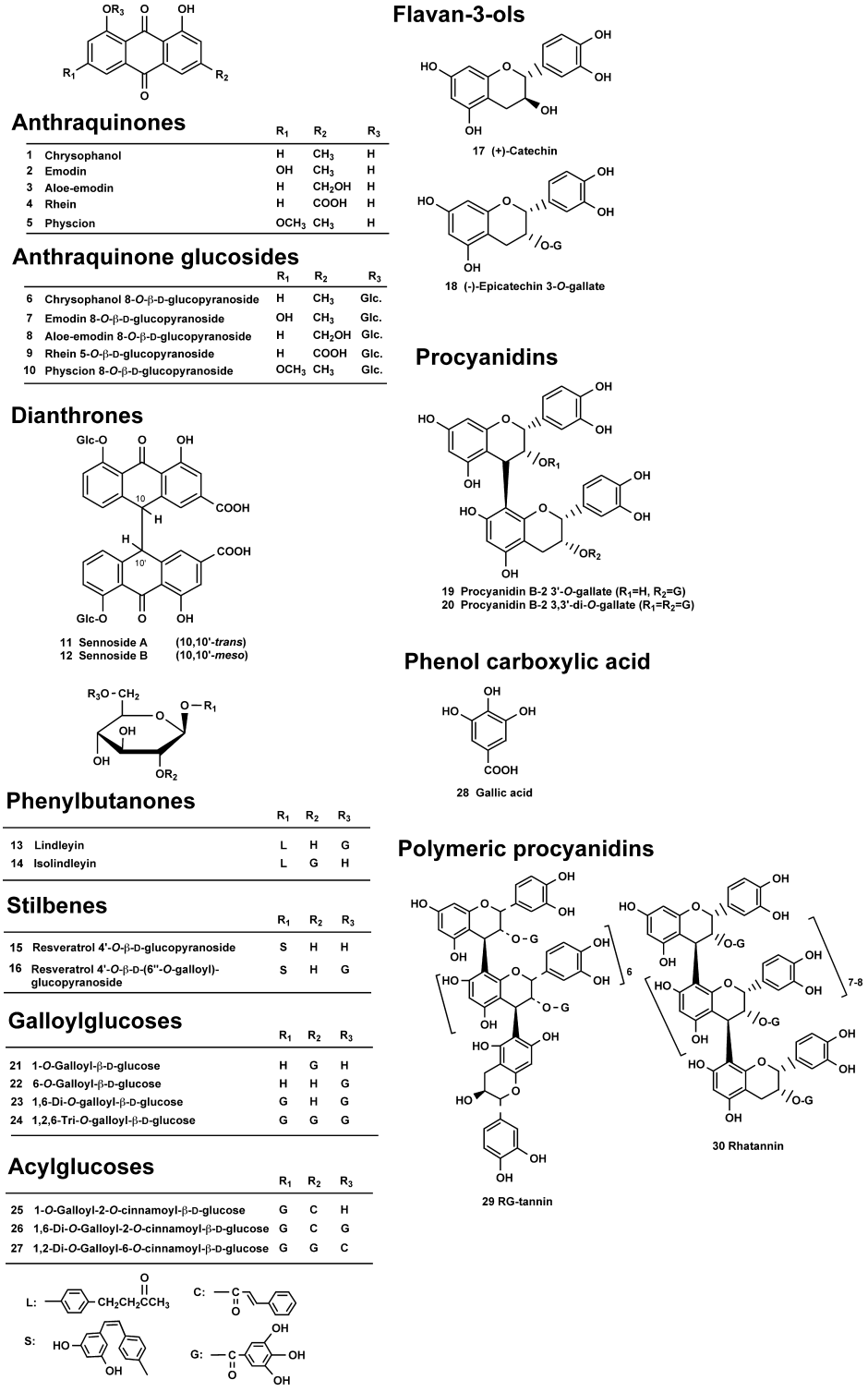
Aliquots (200 mg) of each specimen were extracted three times with 80% acetone (10 ml) at room temperature for 2 h after sonication for 15 min. The organic solvents were combined and evaporated *in vacuo* to give a methanol extract. The extract was dissolved in 10 ml of methanol–water (9 : 1, v/v). After filtration through a 0.2-μm millipore filter, 20 μl of the filtrate was injected into the HPLC system for analysis.

For quantitation of **29** and **30**, 5 ml of the analytical specimen described above was evaporated *in vacuo*. The residue was suspended in 2 ml of methanol then 8 ml of ethyl acetate–dioxane–acetone (85 : 10 : 5, by vol.) was added to the solution. After centrifugation, the precipitate was dissolved in 5 ml of methanol–water (9 : 1, v/v). The solution was filtered through a 0.2-μm millipore filter, and 20 μl of the filtrate was injected into the HPLC system.

Principal Component Analysis The quantitation results of 28 compounds (excluding the data of polymeric procyanidins from Table 3) were subjected principal component analysis (PCA) without preprocessing. All statistical analyses were carried out by Piroruet software (GL Science Inc, Tokyo).

Results and Discussion

Identification of 8 Rhubarb Samples The botanical sources of rhubarb samples Nos. 3, 4, 7, and 18 were determined by ARMS and PCR-RFLP methods as *R. tanguticum*, *R. tanguticum*, *R. palmatum* type I, and *R. palmatum* type II, respectively.[5] By the sequence determination, it was found that the rhizomes of samples Nos. 3 and 4 had identical nucleotide sequences to plant specimens T2 from Huangnan County and T4 from Dulan County of Qinghai Prov., respectively (Table 1, Fig. 1). The sequence of rhizome from sample No. 7 was similar to that of plant specimen Pq1 from Banma County of Qinghai, except for one base substitution at position 379 from the 5′ end of the *mat*K gene. Sample No. 18 had a similar sequence to that of Ps12 from Daofu County of Sichuan, with two base substitutions at positions 259 and 619. The rhizome from sample No. 11 obtained from Sichuan was determined as *R. palmatum* type II, having an identical sequence to plant specimen Pg25 from Gansu. Drug sample No. 23 available on the Japanese market was called “Xiang-huang” and produced in Qinghai, mostly composed of the rhizomes of *R. tanguticum*.[25] However, the rhizome from sample No. 23 was identified as *R. palmatum* type I, showing an identical sequence to plant specimen Pq3 from the southeastern region of Qinghai. In this experiment, two regions of partial *mat*K gene could be amplified to obtain 748- and 539-bp fragments and definitively sequenced, even if the sample was preserved for a long period (No. 24 was collected 25 years ago). The rhizome of this sample was identified as *R. tanguticum*, having a similar sequence to



		R_1	R_2	R_3
1	Chrysophanol	H	CH_3	H
2	Emodin	OH	CH_3	H
3	Aloe-emodin	H	CH_2OH	H
4	Rhein	H	COOH	H
5	Physcion	OCH_3	CH_3	H

		R_1	R_2	R_3
6	Chrysophanol 8-*O*-β-D-glucopyranoside	H	CH_3	Glc.
7	Emodin 8-*O*-β-D-glucopyranoside	OH	CH_3	Glc.
8	Aloe-emodin 8-*O*-β-D-glucopyranoside	H	CH_2OH	Glc.
9	Rhein 5-*O*-β-D-glucopyranoside	H	COOH	Glc.
10	Physcion 8-*O*-β-D-glucopyranoside	OCH_3	CH_3	Glc.

		R_1	R_2	R_3
13	Lindleyin	L	H	G
14	Isolindleyin	L	G	H

		R_1	R_2	R_3
15	Resveratrol 4'-*O*-β-D-glucopyranoside	S	H	H
16	Resveratrol 4'-*O*-β-D-(6''-*O*-galloyl)-glucopyranoside	S	H	G

		R_1	R_2	R_3
21	1-*O*-Galloyl-β-D-glucose	H	G	H
22	6-*O*-Galloyl-β-D-glucose	H	H	G
23	1,6-Di-*O*-galloyl-β-D-glucose	G	H	G
24	1,2,6-Tri-*O*-galloyl-β-D-glucose	G	G	G

		R_1	R_2	R_3
25	1-*O*-Galloyl-2-*O*-cinnamoyl-β-D-glucose	G	C	H
26	1,6-Di-*O*-Galloyl-2-*O*-cinnamoyl-β-D-glucose	G	C	G
27	1,2-Di-*O*-Galloyl-6-*O*-cinnamoyl-β-D-glucose	G	G	C

Fig. 2. Structures of 30 Compounds Used for Quantitative Determination

plant specimen T2 with two base substitutions at positions 619 and 1114.

Quantitative Analysis The extracts of rhubarb samples were quantitatively examined by HPLC method described in our previous paper.[7] Typical chromatographs of the extracts of rhubarb samples derived from different botanical sources are shown in Fig. 3. The drug samples of same botanical origins showed similar chromatographic profiles, whereas the samples derived from different origins showed their own characteristic profiles.

As shown in Table 3, 30 compounds in Rhei Rhizoma of different botanical sources showed considerable variability. As for the total amounts of the 30 compounds, the difference between the highest and lowest ones was >4.4 fold. Sample No. 23 (*R. palmatum* type I obtained from Quinghai) contained the highest amount of the 30 compounds and sample No. 13 (*R. officinale* obtained from Wanyan County, Sichuan) was lowest. The content of sennoside A (**11**), one of

Table 2. Mobile-Phase Gradient Systems A, B, C, and D

System A

Time (min)	0.05 M H_3PO_4	Acetonitrile
0	92	8
50	73	27
60	20	80
70	20	80
80	92	8

System B

Time (min)	0.05 M H_3PO_4	Acetonitrile	Methanol
0	92	8	0
30	77	10	13
50	77	15.3	7.7
60	20	80	0
70	20	80	0
80	92	8	0

System C

Time (min)	0.05 M H_3PO_4	Acetonitrile	Methanol
0	77	10	13
10	77	10	13
40	66.8	26.6	6.8
50	20	70	10
60	77	10	13

System D

Time (min)	0.05 M H_3PO_4	Acetonitrile
0	92	8
25	75	25
35	75	25
50	85	15
75	55	45
85	55	45
90	92	8

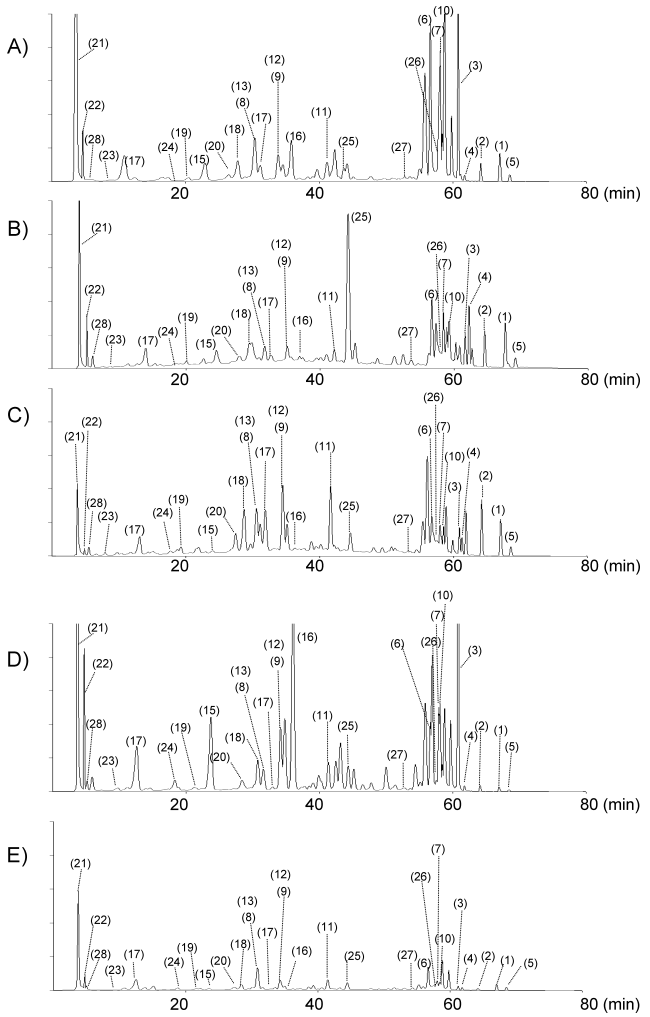


Fig. 3. HPLC Chromatograms of Representative Rhubarb Samples Originating from Type I, Type II, and Type III of *Rheum palmatum*, *R. tanguticum*, and *R. officinale*

A): sample No. 7, *R. palmatum* type I; B): sample No. 20, *R. palmatum* type II; C): sample No. 8, *R. palmatum* type III; D): sample No. 2, *R. tanguticum*; E): sample No. 13, *R. officinale*.

the major components with purgative activity, was >1.0% in rhubarb samples obtained from Jiulong, Litang, and Shimian Counties of Sichuan, whose sources were *R. palmatum* type III. On the other hand, rhubarb samples belonging to *R. palmatum* type II or *R. officinale* obtained from Wanyan County of Sichuan and Li County of Gansu showed lower content of sennoside A (<0.25%). (+)-Catecin (**17**) and resveratrol-glucosides (**15**, **16**), major components for the improvement of blood circulation,[26] were observed in high content in the drug samples from Qinghai, whose botanical sources were *R. tanguticum* or *R. palmatum* type I. Futhermore, lindleyin (**13**), which shows analgesic and anti-inflammatory activities,[27] was revealed higher in samples derived from *R. palmatum* type I.

On the basis of the results of quantitative analysis, the 10-directed radar graphs of 24 rhubarb samples were constructed (Fig. 4). Each direction shows anthraquinones, anthraquinone glucosides, dianthrones, phenylbutanones, stilbenes, flavan-3-ols, procyanidins, galloylglucoses, acylglucoses, and gallic acid. The rhubarbs with the same botanical sources showed similar chemical constituent profiles as follows:

1. Rhubarb derived from *R. tanguticum* collected at Quinghai Prov. showed high contents of anthraquinones and their glycosides, stilbenes, and flavan-3-ols.
2. Rhubarb derived from *R. palmatum* type I in southeastern Quinghai Prov. to northwestern Sichuan Prov. contained relatively large amounts of phenylbutanones and procyanidins compared with those from *R. tanguticum* (most constituents except gallic acid were contained in this type).
3. Rhubarb derived from *R. palmatum* type III in the central to western area of Sichuan Prov. contained large quantities of dianthrones and anthraquinone glycoside compared with other constituents.
4. Rhubarb derived from *R. officinale* in Sichuan Prov. and *R. palmatum* type II in Sichuan, Yunnan, and Gansu Provs. contained small amounts of dianthrones. Among them, rhubarb produced in Li County, Gansu Prov. was characterized by a relatively high content of acylglucoses.

Table 3. Contents of 30 Chemical Components in Rhubarb Samples (Mean, $n=3$) (mg/g)

Compound	Market	Qinghai Prov.						Sichuan Prov.					
	Sample No.	1	2	3	4	5	6	7	8	9	10	11	12
	Botanical origin[a)]	RPI	RT	RT	RT	RT	RT	RPI	RPIII	RPIII	RPIII	RPII	RPII
	TMPW No.	20061	20065	20066	20107	20108	20109	20218	20216	20574	20573	20576	20266
	Weight of extract (g)	476.33	451.33	242.83	398.83	300.50	338.00	470.66	315.00	290.00	261.83	301.66	357.50
1. Anthraquinones													
Chrysophanol (**1**)		1.80	0.76	1.89	0.93	0.98	2.68	2.85	3.56	2.40	2.07	3.50	3.55
Emodin (**2**)		0.02	0.01	0.47	0.02	0.49	1.51	0.64	0.54	0.00	1.97	0.09	0.03
Aloe-emodin (**3**)		5.99	20.91	15.35	19.99	14.85	22.99	7.23	1.12	0.52	0.75	0.12	0.68
Rhein (**4**)		2.66	0.01	1.10	0.01	0.01	1.14	0.36	3.70	1.84	1.23	0.89	0.01
Physcion (**5**)		1.10	0.67	1.43	0.87	1.63	2.32	1.93	2.59	4.35	1.32	2.97	2.42
Subtotal		11.57	22.36	20.24	21.82	17.96	30.64	13.01	11.51	9.11	7.34	7.57	6.69
2. Anthraquinone glucosides													
Chrysophanol 8-*O*-*β*-D-glucopyranoside (**6**)		7.08	6.17	5.75	7.05	7.18	12.24	9.79	9.42	3.78	1.48	9.79	9.44
Emodin 8-*O*-*β*-D-glucopyranoside (**7**)		4.18	9.90	5.44	6.57	5.35	7.55	6.78	4.32	6.39	4.28	3.76	5.90
Aloe-emodin 8-*O*-*β*-D-glucopyranoside (**8**)		2.37	7.03	1.05	4.96	3.60	0.28	3.44	1.83	0.39	0.19	0.60	3.63
Rhein 5-*O*-*β*-D-glucopyranoside (**9**)		11.52	9.34	3.93	12.06	8.58	3.16	9.30	9.26	7.46	10.04	0.95	0.24
Physcion 8-*O*-*β*-D-glucopyranoside (**10**)		0.25	2.97	1.14	4.31	3.71	5.67	3.36	0.34	2.52	1.75	1.45	3.02
Subtotal		25.40	35.41	17.31	34.95	28.42	28.90	32.67	25.17	20.54	17.74	16.55	22.23
3. Dianthrones													
Sennoside A (**11**)		5.06	6.69	5.87	6.79	4.33	8.71	4.03	12.16	13.62	10.68	1.70	2.05
Sennoside B (**12**)		1.37	3.69	1.04	3.28	2.44	5.74	1.23	4.48	5.06	4.12	0.02	0.19
Subtotal		6.43	10.38	6.91	10.07	6.77	14.45	5.26	16.64	18.68	14.80	1.72	2.24
4. Phenylbutanones													
Lindleyin (**13**)		18.12	2.46	5.65	4.15	4.86	3.77	10.85	6.39	2.46	8.61	0.65	3.56
Isolindleyin (**14**)		3.63	3.91	6.49	4.33	6.59	0.70	5.66	4.49	1.89	1.24	1.04	1.13
Subtotal		21.75	6.37	12.14	8.48	11.45	4.47	16.51	10.88	4.35	9.85	1.69	4.69
5. Stilbenes													
Resveratrol 4′-*O*-*β*-D-glucopyranoside (**15**)		4.58	9.97	2.68	5.27	1.43	6.72	3.71	4.11	11.64	0.87	1.02	1.01
Resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**16**)		6.21	21.01	6.41	13.56	15.02	13.41	5.56	1.35	1.15	0.24	0.01	0.01
Subtotal		10.79	30.98	9.09	18.83	16.45	20.13	9.27	5.46	12.79	1.11	1.03	1.02
6. Flavan-3-ols													
(+)-Catechin (**17**)		13.92	19.40	N.D.	33.59	19.79	17.20	9.46	5.77	10.69	13.34	0.02	N.D.
(−)-Epicatechin 3-*O*-gallate (**18**)		6.42	2.37	4.71	2.18	1.99	3.38	3.39	4.66	6.52	5.86	11.18	6.19
Subtotal		20.34	21.77	4.71	35.77	21.78	20.58	12.85	10.43	17.21	19.20	11.20	6.19
7. Procyanidins													
Procyanidin B-2 3′-*O*-gallate (**19**)		2.50	1.36	5.93	0.87	1.69	1.40	1.01	1.22	4.88	4.84	0.48	0.58
Procyanidin B-2 3,3′-di-*O*-gallate (**20**)		6.30	0.86	0.75	1.52	2.66	0.96	2.79	7.49	5.34	5.14	2.20	2.22
Subtotal		8.80	2.22	6.68	2.39	4.35	2.36	3.80	8.71	10.22	9.98	2.68	2.80
8. Galloylglucoses													
1-*O*-Galloyl-*β*-glucose (**21**)		11.02	21.36	7.99	34.01	20.38	15.60	8.50	6.01	5.54	6.91	1.88	11.58
6-*O*-Galloylglucose (**22**)		1.18	4.91	4.08	5.93	4.13	2.03	1.69	2.25	0.56	0.34	3.27	10.78
1,6-Di-*O*-Galloyl-*β*-D-glucose (**23**)		N.D.	0.71	N.D.	1.69	0.13	0.50	0.12	0.03	0.02	0.13	1.71	N.D.
1,2,6-Tri-*O*-Galloyl-*β*-D-glucose (**24**)		0.78	0.75	N.D.	1.82	0.39	0.54	0.23	0.54	1.08	0.89	0.40	0.33
Subtotal		12.98	27.73	12.07	43.45	25.03	18.67	10.54	8.83	7.20	8.27	7.26	22.69
9. Acylglucoses													
1-*O*-Galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**25**)		1.62	2.34	2.07	4.40	0.86	1.07	0.46	0.71	1.02	1.20	1.86	1.05
1,6-Di-*O*-Galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**26**)		0.16	1.37	0.69	3.63	2.40	5.34	1.26	0.99	1.70	0.74	0.50	0.06
1,2-Di-*O*-Galloyl-6-*O*-cinnamoyl-*β*-D-glucose (**27**)		0.59	0.40	0.40	0.73	0.36	0.47	0.41	0.09	0.25	0.35	0.54	0.31
Subtotal		2.37	4.11	3.16	8.76	3.62	6.88	2.13	1.79	2.97	2.29	2.90	1.42
10. Gallic acid (**28**)		1.07	0.73	2.33	1.78	1.40	1.30	0.58	1.91	0.48	0.47	0.85	2.86
11. Polymeric procyanidins													
RG-tannin (**29**)		44.99	31.48	19.53	23.33	34.79	38.27	50.91	34.85	35.54	17.77	24.34	22.91
Rhatannin (**30**)		1.90	0.05	6.43	0.05	0.99	2.55	1.68	8.45	4.27	2.43	2.63	2.05
Subtotal		46.89	31.53	25.96	23.38	35.78	40.82	52.59	43.30	39.81	20.20	26.97	24.96
Total		168.39	193.59	120.60	209.68	173.01	189.20	159.21	144.63	143.36	111.25	80.42	97.79

To facilitate the classification of rhubarb drugs, principal component analysis was applied to the quantitation results of 28 compounds. The first three principal components, which accounted for 88.2% of the total content, were evaluated in this study. The results on loading indicated that aole-emodin (**3**), rhein 5-*O*-*β*-D-glucopyranoside (**9**), sennoside A (**11**), lindleyin (**13**), resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**16**), (+)-catechin (**17**), and 1-*O*-galloyl-*β*-glucose (**21**) contributed to the first PC; aole-emodin, rhein 5-*O*-*β*-D-glucopyranoside, sennoside A, lindleyin, (−)-epicatechin 3-*O*-gallate (**18**), procyanidin B-2 3,3′-di-*O*-gallate (**20**), and 1-*O*-galloyl-*β*-glucose to the second PC; and aole-emodin, chrysophanol 8-*O*-*β*-D-glucopyranoside (**6**), emodin 8-*O*-*β*-D-glucopyranoside (**7**), resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**16**), 1-*O*-galloyl-*β*-glucose, and 1-*O*-galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**25**) to the third PC.

The score plot of chemical component data from 24 rhubarb samples is shown in Fig. 5. The plot shows that the

Table 3. (Continued)

Compound	Market	Sichuan Prov.		Yunnan Prov.	Gansu Prov.			Japan					Hong Kong
	Sample No.	13	14	15	16	17	18	19	20	21	22	23	24
	Botanical origin[a)]	RO	RPII	RPII	RPII	RPII	RPIII	RPIII	RPII	RPI	RT	RPI	RT
	TMPW No.	20267	7422	19535	20926	20928	20932	19928	19927	19948	19949	19929	414
	Weight of extract (g)	174.50	244.50	301.17	433.80	405.00	308.33	407.50	401.33	316.50	274.83	461.66	308.00
1. Anthraquinones													
Chrysophanol (**1**)		0.87	1.35	1.78	2.25	2.77	1.18	4.43	2.12	2.36	2.10	0.73	1.38
Emodin (**2**)		N.D.	0.01	0.03	0.03	0.02	0.02	2.69	0.02	0.89	0.03	0.01	0.03
Aloe-emodin (**3**)		N.D.	0.01	0.01	2.55	0.47	11.57	0.02	0.73	0.39	7.22	6.22	20.56
Rhein (**4**)		0.01	N.D.	0.51	2.32	1.91	0.01	2.04	0.65	3.57	2.89	0.02	0.44
Physcion (**5**)		0.89	1.01	2.03	2.37	2.15	1.27	2.73	1.74	3.21	2.34	0.62	2.38
Subtotal		1.77	2.38	4.36	9.52	7.32	14.05	11.91	5.26	10.42	14.58	7.60	24.79
2. Anthraquinone glucosides													
Chrysophanol 8-*O*-*β*-D-glucopyranoside (**6**)		3.92	6.93	2.58	2.65	4.75	8.88	5.90	5.35	3.81	5.77	2.09	9.54
Emodin 8-*O*-*β*-D-glucopyranoside (**7**)		2.22	6.00	4.18	10.49	6.77	8.45	3.35	9.89	2.87	8.14	4.45	4.41
Aloe-emodin 8-*O*-*β*-D-glucopyranoside (**8**)		3.15	1.09	1.01	1.04	1.19	4.62	5.18	1.82	3.24	2.36	6.23	13.28
Rhein 5-*O*-*β*-D-glucopyranoside (**9**)		0.95	0.77	0.61	0.52	0.68	5.31	6.25	0.01	5.57	4.73	24.54	10.19
Physcion 8-*O*-*β*-D-glucopyranoside (**10**)		0.05	2.21	1.42	1.44	2.15	5.73	0.34	0.82	0.05	2.59	0.05	5.91
Subtotal		10.29	17.00	9.80	16.14	15.54	32.99	21.02	17.89	15.54	23.59	37.36	43.33
3. Dianthrones													
Sennoside A (**11**)		1.78	1.77	2.56	1.77	0.79	2.98	5.82	3.15	9.65	4.22	9.39	6.63
Sennoside B (**12**)		N.D.	N.D.	0.75	0.44	0.08	0.38	1.58	1.02	N.D.	1.36	1.29	2.17
Subtotal		1.78	1.77	3.31	2.21	0.87	3.36	7.40	4.17	9.65	5.58	10.68	8.80
4. Phenylbutanones													
Lindleyin (**13**)		4.61	5.79	1.79	1.71	6.02	2.77	0.60	6.26	10.73	2.09	20.61	10.36
Isolindleyin (**14**)		0.46	1.47	0.69	2.56	3.23	4.42	3.43	3.09	6.88	5.98	14.68	1.98
Subtotal		5.07	7.26	2.48	4.27	9.25	7.19	4.03	9.35	17.61	8.07	35.29	12.34
5. Stilbenes													
Resveratrol 4′-*O*-*β*-D-glucopyranoside (**15**)		0.81	1.79	1.53	1.64	1.82	3.53	0.92	2.38	4.69	3.84	6.14	5.36
Resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**16**)		0.01	0.20	0.01	0.01	0.02	3.08	0.78	0.18	2.54	10.70	18.60	10.63
Subtotal		0.82	1.99	1.54	1.65	1.84	6.61	1.70	2.56	7.23	14.54	24.74	15.99
6. Flavan-3-ols													
(+)-Catechin (**17**)		9.27	N.D.	0.33	12.97	10.05	12.32	5.59	11.64	7.81	18.16	31.30	N.D.
(−)-Epicatechin 3-*O*-gallate (**18**)		1.18	2.01	0.92	1.17	1.54	1.91	2.59	1.82	6.08	0.89	11.01	1.29
Subtotal		10.45	2.01	1.25	14.14	11.59	14.23	8.18	13.46	13.89	19.05	42.31	1.29
7. Procyanidins													
Procyanidin B-2 3′-*O*-gallate (**19**)		0.81	0.53	2.32	0.60	1.28	1.15	1.07	2.28	3.68	3.82	3.63	0.56
Procyanidin B-2 3,3′-di-*O*-gallate (**20**)		0.85	1.95	1.79	1.75	1.03	4.16	2.05	2.82	7.90	N.D.	11.65	0.68
Subtotal		1.66	2.48	4.11	2.35	2.31	5.31	3.12	5.10	11.58	3.82	15.28	1.24
8. Galloylglucoses													
1-*O*-Galloyl-*β*-glucose (**21**)		3.99	18.46	4.05	10.55	12.88	8.01	3.51	10.44	3.97	11.05	10.16	7.88
6-*O*-Galloylglucose (**22**)		0.95	5.18	1.57	2.56	3.20	5.05	1.57	1.78	0.05	2.35	2.75	4.65
1,6-Di-*O*-Galloyl-*β*-D-glucose (**23**)		1.39	N.D.	N.D.	0.01	N.D.	0.22	0.05	0.07	N.D.	0.43	0.07	N.D.
1,2,6-Tri-*O*-Galloyl-*β*-D-glucose (**24**)		0.49	0.22	0.31	0.51	0.67	1.13	1.00	0.54	0.59	1.63	1.83	N.D.
Subtotal		6.82	23.86	5.93	13.63	16.75	14.41	6.13	12.83	4.61	15.46	14.81	12.53
9. Acylglucoses													
1-*O*-Galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**25**)		0.06	4.34	0.12	9.19	10.53	0.52	0.06	10.66	1.74	1.47	1.02	1.78
1,6-Di-*O*-Galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**26**)		0.04	N.D.	0.56	0.03	2.39	0.05	0.94	0.02	N.D.	0.03	2.75	0.07
1,2-Di-*O*-Galloyl-6-*O*-cinnamoyl-*β*-D-glucose (**27**)		0.13	0.27	0.96	0.40	0.41	1.15	0.33	0.81	0.05	0.21	0.16	1.07
Subtotal		0.23	4.61	1.64	9.62	13.33	1.72	1.33	11.49	1.79	1.71	3.93	2.92
10. Gallic acid (**28**)		0.69	0.49	0.47	0.81	2.21	3.06	1.47	1.36	1.56	0.94	1.21	0.71
11. Polymeric procyanidins													
RG-tannin (**29**)		17.36	15.39	34.73	26.49	28.93	19.40	35.39	52.54	42.78	27.37	55.57	27.73
Rhatannin (**30**)		0.04	0.06	0.84	0.25	3.56	0.19	6.86	9.53	7.73	2.78	0.02	0.01
Subtotal		17.40	15.45	35.57	26.74	32.49	19.59	42.25	62.07	50.51	30.15	55.59	27.74
Total		56.98	79.30	70.46	101.08	113.50	122.52	108.54	145.54	144.39	137.49	248.80	151.68

a) RT: *R. tanguticum*, RPI: *R. palmatum* type I, RPII: *R. palmatum* type II, RPIII: *R. palmatum* type III, RO: *R. officinale*.

distribution of original *Rheum* species (*R. tanguticum*, 3 intraspecies groups of *R. palmatum*, and *R. officinale*) belonging to different classes could be observed. Sample No. 24, which was collected 25 years ago, showed less content of flavan-3-ols. It has been reported that these polyphenolic compounds receive atmospheric oxidation readily during the air-drying process.[28)] As for the drying procedure of commercially available products, we obtained data showing that samples No. 9 and No. 11 were air-dried in a dark place, and sample No. 10 was air-dried in a dark place followed by heat drying. Samples No. 9 and No. 10, which were the same genetic type (*R. palmatum* type III) produced in neighboring counties, but processed with different procedures, showed similar patterns of chemical constituents. On the other hand, samples No. 10 and No. 11, which were of different genetic type but produced in the same county, provided different

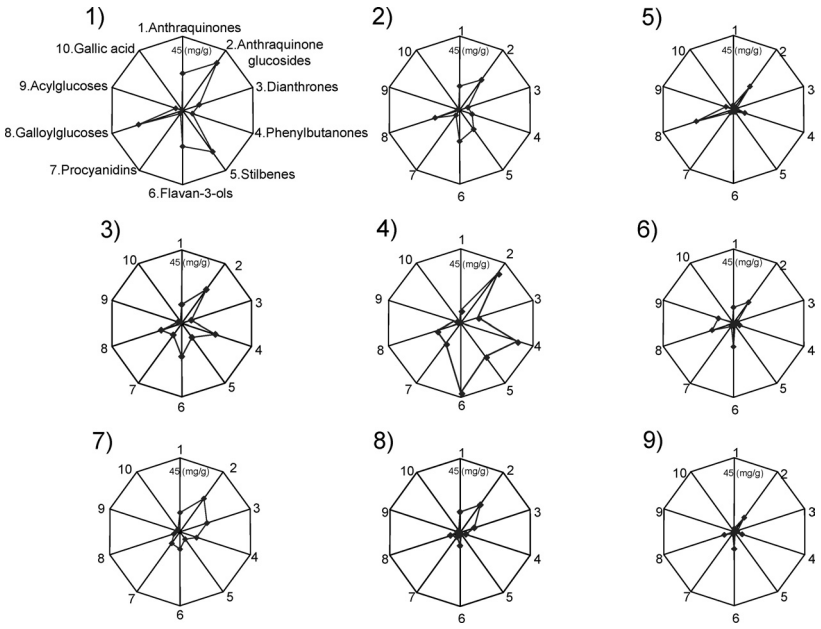


Fig. 4. Ten-Direction Radar Graphs of Rhubarb Samples Originating from Type I, Type II, and Type III of *Rheum palmatum*, *R. tanguticum*, and *R. officinale*

1): sample No. 2, *R. tanguticum*; 2): sample No. 22, *R. tanguticum*; 3): sample No. 1, *R. palmatum* type I; 4): sample No. 23, *R. palmatum* type I; 5): sample No. 14, *R. palmatum* type II; 6): sample No. 16, *R. palmatum* type II; 7): sample No. 8, *R. palmatum* type III; 8): sample No. 19, *R. palmatum* type III; 9): sample No. 13, *R. officinale*.

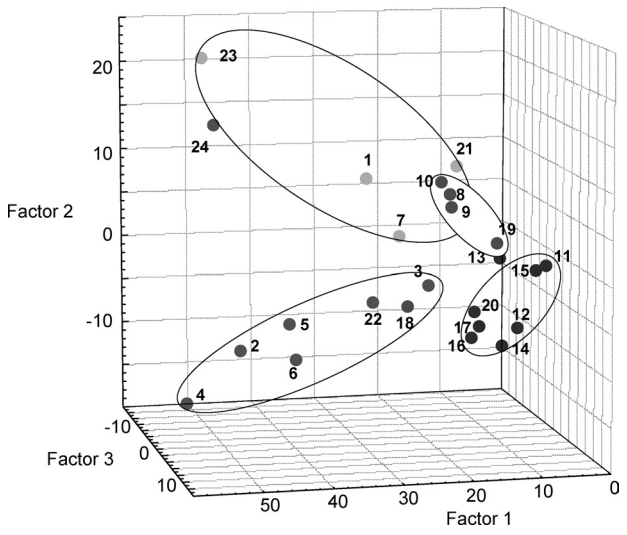


Fig. 5. Principal Component Analysis Score Plot of Chemical Component Data from 24 Rhubarb Samples

chemical component patterns. Although further investigation is required, these results suggest that genetic factors of rhubarb are extremely important for chemical component patterns of 28 compounds. On the other hand, regarding the contents of RG-tannin and rhatannin there was no relation to the botanical sources of rhubarb, as shown in Table 3. It has been reported that different processing methods for drying such as burning with fire or freeze-drying of high altitude might affect their contents.[29)]

Kashiwada *et al.* reported that rhubarb produced in Sichuan Prov. can be classified into two groups depending on the relative concentration of phenylbutanones, stilbenes, and procyanidines to those of other constitutents.[3)] Our results suggest that there are four kinds of rhubarb in Sichuan: one kind originating from *R. palmatum* type III contains large amounts of dianthrones, anthraquinone glucosides, and anthraquinones; one kind from *R. palmatum* type I shows high content of phenylbutanones, stillbenes, procyanidins, and flavan-3-ols besides the above three chemical groups; one kind from *R. palmatum* type II contains small amounts of dianthrones, stilbenes, and others; and lastly one kind from *R. officinale* contains a smaller amount of anthraquinones. Regarding rhubarbs produced in Qinghai and Gansu Provs., there are two kinds. Qinghai productions were derived from *R. tanguticum* and *R. palmatum* type I, and Gansu productions from *R. palmatum* type II and *R. palmatum* type III.

In conclusion, each Rhei Rhizoma derived from different taxa, genotype, and production sites showed a characteristic chromatographic profile and comparable specific shape in the 10-directed radar graphs constructed on the basis of the contents of 28 major pharmacological active constituents. Furthermore, a relationship between chemical constituent patterns and genetic varieties of rhubarb samples was indicated. At the same time, the characteristic constituent pattern of Rhei Rhizoma with different origins gave useful information on proper use of rhubarb according to different therapeutic purposes.

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