Studies on rhubarb (Rhei Rhizoma). VII. Evaluation of rhubarbs by high-performance liquid chromatography

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Abstract

Simple and convenient new procedures have been developed for the simultaneous determination of μmol levels of all the ingredients in rhubarbs by high-performance liquid chromatography. Neither preliminary fractionation nor complicated chromatographic conditions were involved, and the methods allow clear separation of almost all the compounds including anthraquinones, sennosides, lindleyins, stilbenes, low-molecular-weight tannins, *etc.* Application of these methods to the evaluation of various types of commercially available Chinese rhubarbs has led to the conclusion that the composition and contents of the components differ markedly even in those with the same commercial names.

Keywords rhubarb, *Rheum* sp., Polygonaceae, evaluation, high-performamce liquid chromatography

Abbreviations HPLC ; high - performance liquid chromatography, JP ; the Phamacopoea of Japan, Batei Daiô (Mar-Tie-Huang) ; 馬蹄大黄, Gaô (Ija-Huang) ; 雅黄, Kinmon Daiô (Jing-Wuen-Da-Huang) ; 錦紋大黄

Introduction

The roots and rhizomes of various species of rhubarbs (*Rheum* sp.), most of which originally grow in the Asiatic regions, have been utilized for medicinal purposes. In Europe, the rhubarbs are used exclusively as a purgative. However, in traditional Chinese herbal therapy, they have been used solely or in combination with other crude drugs not only as a purgative but also for the treatments of many diseases such as a blood-stasis syndrome, hypertension, mental and renal disorders, urticaria, eczema, *etc.*[1)] In addition, recent biological and pharmacological studies have revealed that rhubarbs possess a remarkable urea - nitrogen (BUN)- decreasing activity,[2-4)] together with a curative effect on chronic renal failure[5-7)] and inhibitory effects on angiotensin-converting enzyme (ACE).[8,9)] In parallel with these biological studies, the chemical examination of rhubarbs is currently under way to elucidate the active ingredients. We previously reported the isolation and characterization of more than ninety compounds from a variety of Chinese rhubarbs,[10-17)] and demonstrated that, among others, lindleyin, a gallate of *p*-hydroxyphenyl-butanone glucoside, possesses analgesic and anti-inflammatory activities almost equal to those of aspirin and phenylbutazone,[4)] while rhatannins are

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the active components to decrease urea-nitrogen concentrations in serum.[2,3] During the course of these chemical works, we have noticed the heterogeneity (in both quantitative and qualitative senses) of components in various Chinese rhubarbs, even in those with the same commercial names. This fact strongly indicates that the above-mentioned biological activities and efficacy as a drug differ in types of rhubarbs. The purpose of this study is to establish the method for evaluating rhubarbs by means of high-performance liquid chromatography (HPLC), and also to extend this method to analysis of commercially available rhubarbs.

Materials and Methods

Materials—— About half of the rhubarbs used for HPLC analysis were purchased from the market in Hong Kong. Other were provided by

Table I Rhubarb specimens examined

	Commercial name	Place of production or place purchased	Date purchased
1	Kinmon Daiô (錦紋大黄)		1983.5
2	Kinmon Daiô (Grade A)	Hong Kong (market)	
3	Kinmon Daiô (Grade B)	Hong Kong	
4	Kinmon Daiô (Merck)		
5	Kinmon Daiô		
6	Chokichiô (長吉黄)	Hong Kong	1983.5
7	Chukichiô (中吉黄)	Hong Kong	1983.5
8	Gaô (雅黄)	Hong Kong	1983.5
9	Gaô (Grade II)	Hong Kong	1983.5
10	Gaô (Class II)	Hong Kong	
11	Gaô (Class III)	Hong Kong	
12	Gaô (Grade C)		
13	Gaô		1972.5
14	Batei Daiô (馬蹄大黄)	Hong Kong	1983.6
15	Batei Daiô	Hong Kong	1983.7
16	Batei Daiô	Hong Kong	1983.5
17	Grade I	Si-Chuan (produced)	1982.11
18	Grade II		1983.5
19	Tôgai (等外)	Si-Chuan	1983.6
20	Kon-Ô (根黄)	Si-Chuan	1983.10
21	Grade II	Si-Chuan	1983.10
22	Grade III	Si-Chuan	1983.10
23	Tôgai no Ge (等外の下)		1983.11
24	Grade III	Hong Kong	1983.6
25	Tôgai (等外)	Hong Kong	1983.9
26	Tôgai		
27	Unknown		
28	Unknown		
29	Unknown		
30	Imo Daiô (芋大黄)	Hong Kong	1983.5
31	Chôsen Daiô (朝鮮大黄)		1971.5
32	Hokkai Daiô (北海大黄)		
33	Daiô (JP) (Uchida)		
34	Daiô (JP) (Mikuni)		

Nihon Funmatsu Co., Ltd., Tsumura Jyuntendo Co., Ltd., and Mikuni Shôten Co., Ltd. The commercial names, origins, *etc.* are listed in Table I. Standard samples for analysis were obtained previously from various types of rhubarbs,[4,10-17] and are shown in Table II.

Chromatographic Conditions —— (i) The high-performance liquid chromatograph consisted of a Toyo Soda SP-8700 solvent delivery system, a UV-8 model II spectrometer, and a Nucleosil $5C_{18}$ (Macherey-Nagel) column (4 mm i.d. × 300 mm). The column temperature was at 40℃ and the detection at a wavelength at 280 nm. Elution was performed at a flow rate of 1.0 ml/min with increasing amounts of acetonitrile in 0.05M NaH_2PO_4 buffer solution (see Fig. 1). (ii) The high-performance liquid chromatographic system consisted of a Hitachi model 655 liquid chromatograph, a Rheodyne 1725 injection valve, a Hitachi variable wavelength spectrometric detector, and a Nucleosil $5C_{18}$ (Macherey-Nagel) column (4 mm i. d. × 25 mm). The mobile phase

Table II Standard samples

NO.	Compound	Reference
1	gallic acid	
2	6-*O*-galloylglucose	16
3	1-*O*-galloy-*β*-D-glucose	16
4	(+)-catechin 5-*O*-*β*-D-glucoside	17
5	gallic acid 3-*O*-*β*-D-(6′-*O*-galloyl)-glucoside	16
6	gallic acid 4-*O*-*β*-D-(6′-*O*-galloyl)-glucoside	16
7	(+)-catechin	11
8	1,6-di-*O*-galloyl-*β*-D-glucose	16
9	4-(*p*-hydroxypheny)-butanone glucoside	4
10	(−)-epicatechin	4
11	1,2,6-tri-*O*-galloyl-*β*-D-glucose	4
12	procyanidin B-1 3-*O*-gallate	4
13	sennoside B	
14	rhein 8-*O*-*β*-D-glucoside	10
15	3,4′, 5-trihydroxystilbene 4′-*O*-*β*-D-glucoside	11
16	sennoside A	
17	piceatannol 3′-*O*-*β*-D-glucoside	15
18	(−)-epicatechin 3-*O*-gallate	4
19	procyanidin B-2 3,3′-di-*O*-gallate	4
20	lindleyin	4
21	aloe-emodin 8-*O*-*β*-D-glucoside	10
22	isolindleyin	16
23	rhaponticin	15
24	3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucoside	11
25	6-hydroxymusizin 8-*O*-*β*-D-glucoside	12
26	2′-*O*-cinnamoylglucogallin	13
27	rhein	10
28	torachrysone 8-*O*-*β*-D-glucoside	12
29	desoxyrhaponticin	15
30	chrysophanol 1-and 8-*O*-*β*-D-glucoside	10
31	emodin 8-*O*-*β*-D-glucoside	10
32	physcion 8-*O*-*β*-D-glucoside	10
33	emodin	10
34	chrysophanol	10

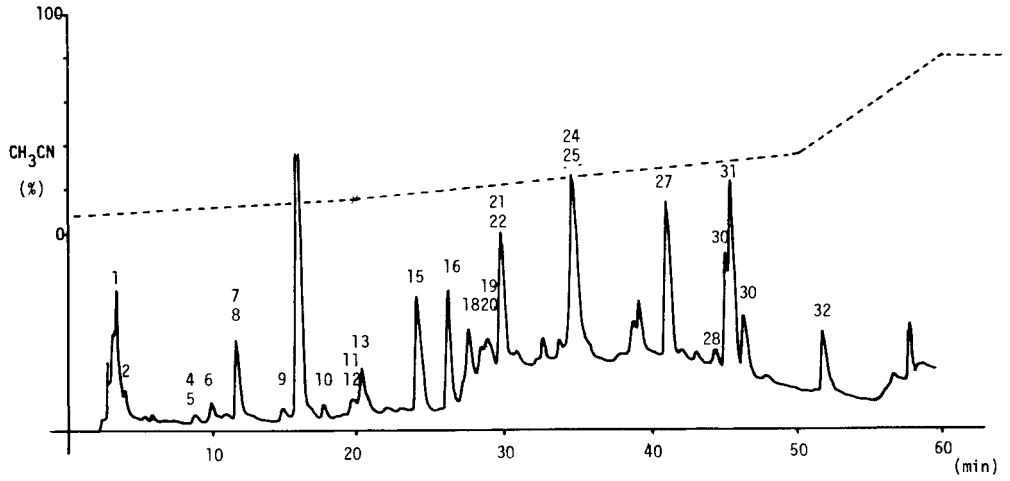

Fig. 1 High-preformance liquid chromatogram of rhubarb (JP) (Uchida).
Numbers on the peaks correspond to those of the compounds listed in Table II. Conditions : (i)

was (a) acetonitrile-0.05M $NH_4H_2PO_4$ (pH 2.5) (4 : 1), (b) acetonitrile-methanol-water (13 : 13 : 74) (oxalic acid 1g/l), or (c) acetonitrile-water (oxalic acid 1g/l) (8 : 92). Elution was performed at ambient temperature at a flow rate of 1.2 ml/min, and the detection at either 280 nm, 350 or 420 nm. Quantitative determination was made by the peak area method using a combination of a Hitachi 855 data processor and a Shimadzu chromatopak C-RIA instrument. For the identification of some of the anthraquinones and sennosides, a Shimadzu photodiode-alley detector, model SPD-MID was used. Standard curves were prepared by the conventional method.

Sample Preparation —— (A) For the chromatographic condition (i) ; finely powdered rhubarb (0.4 g) was extracted three times (each 24 h) with acetone-water (4 : 1) (each 20 ml) at room temperature. The solvent was evaporated under reduced pressure. The residue was treated with methanol (20 ml) and the insoluble materials were filtered off through a milipore filter. A portion (5 μl) of the filtrate was injected into HPLC. (B) For the chromatographic condition (ii) ; finely ground rhubarb (1.0 g) in acetone-water (1 : 1) (20 ml) was shaken for 15 min at room temperature. The supernatant was separated by centrifugation for 5 min at 3000 rpm. This procedure was repeated once, and the residue was finally washed with acetone. The extracts and washing were combined, and the volume was adjusted to 50 ml by diluting with acetone. A portion (10 ml) was partitioned three times with ethyl acetate (10 ml each). The ethyl acetate layer was dried over Na_2SO_4 and evaporated under reduced pressure to furnish a residue, which was dissolved in a mixture of acetonitrile (9.5 ml) and water (0.5 ml), and subjected to HPLC analysis [conditions (ii a) and (ii b)]. The above aqueous layer was concentrated to about half volume by evaporation under reduced pressure, diluted to 5 ml by adding acetonitrile (0.5 ml) and water, and subjected to HPLC analysis [condition (ii c)].

Results

Four typical examples of high-performance liquid chromatograms analyzed by the condition (i) are illustrated in Fig. 2. The numbers on the peaks correspond to those of compounds listed in Table II, and the peaks blacked out indicate those identified by comparisons of their retention times and by co-chromatography. As is evident from these chromatograms, five couples of compounds, *i.e.*, (+)-catechin 5-*O*-β-D-glucoside (4) and gallic acid 3-*O*-β-D-(6′-*O*-galloyl)-glucoside (5), 1,2,6-tri-*O*-galloyl-β-D-glucose (11) and procyanidin B-1 3-*O*-gallate (12), isolindleyin (21)

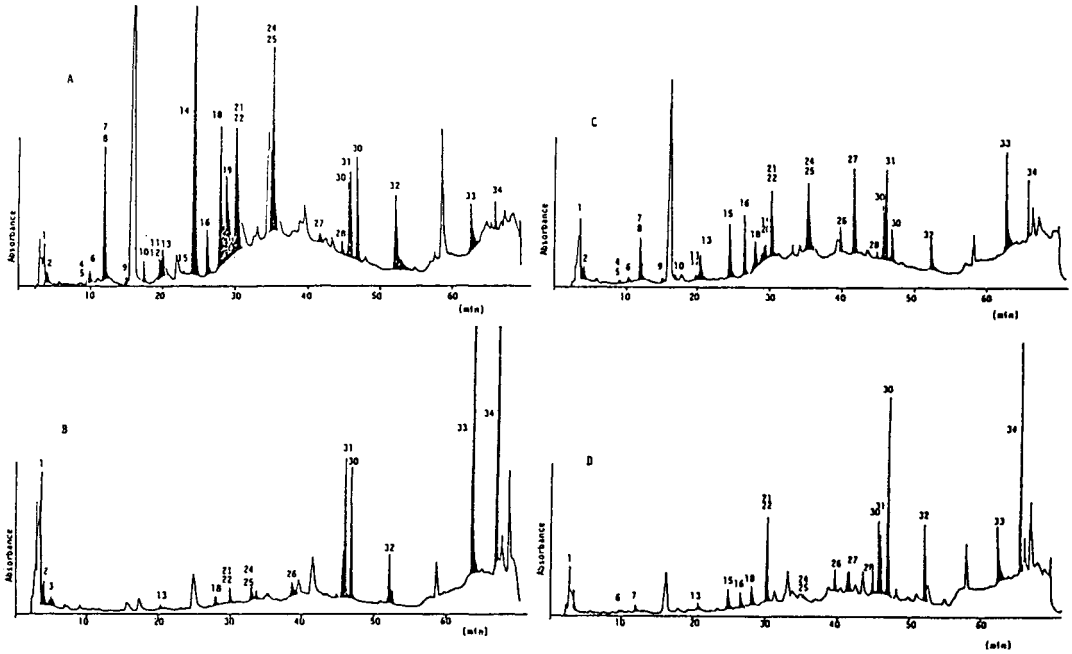

Fig. 2 High-performance liquid chromatograms of A) Kinmon Daiô (grade A), B) Kinmon Daiô (grade B), C) Gaô and D) Batei Daiô.
Conditions : (i).

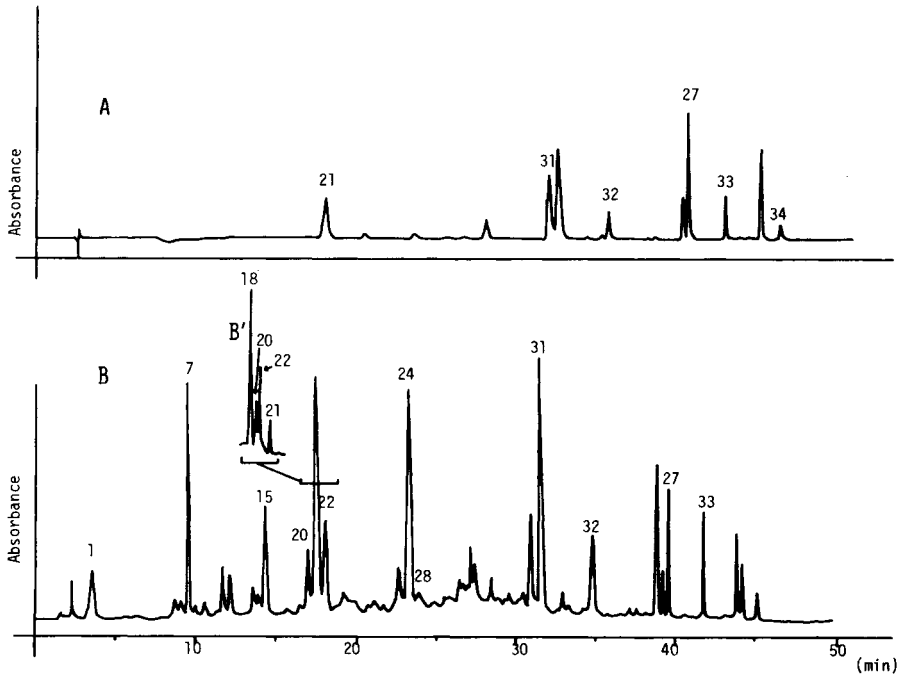

Fig. 3 High-performance liquid chromatograms of the ethyl acetate-soluble portion.
A) rhubarb (grade I) at 420 nm. B) rhubarb (grade I) at 280 nm.
Conditions : A) and B), (ii a) ; B^1), (ii b)

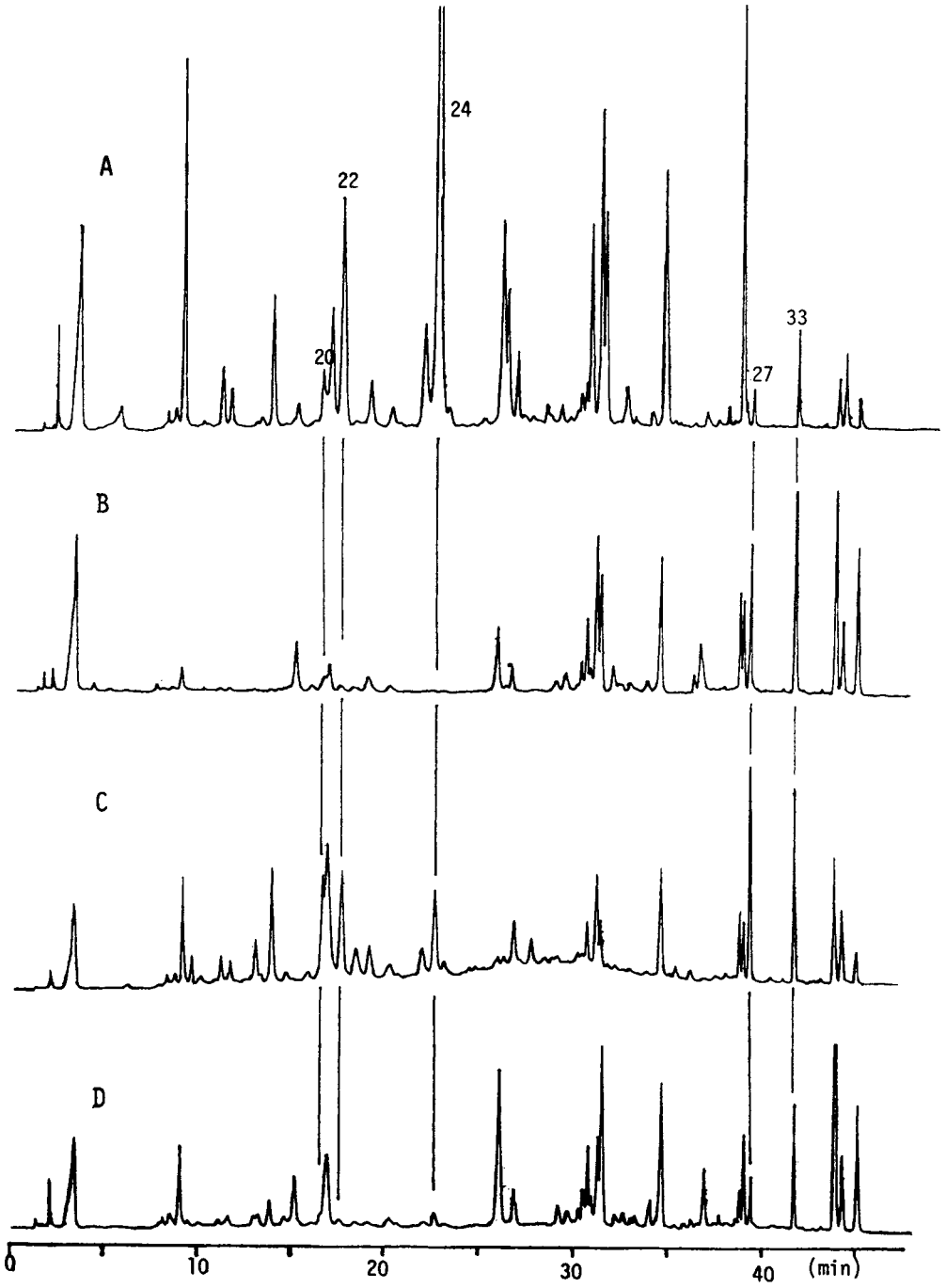

Fig. 4 High-performance liquid chromatograms of the ethyl acetate-soluble portion (at 280 nm). A) Kinmon Daiô (grade A), B) Kinmon Daiô (grade B), C) Gaô, D) Batei Daiô. Conditions : (ii a)

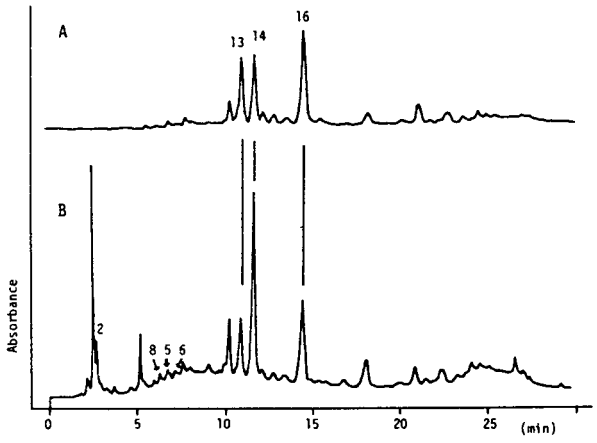

Fig. 5 High-performance liquid chromatograms of the aqueous layer (Grade I).
A : at 350 nm, B : at 280 nm. Conditions : (ii c).

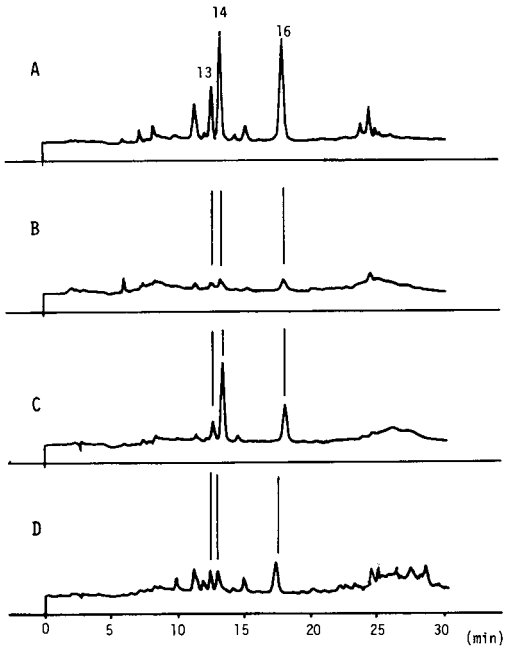

Fig. 6 High-performance liquid chromatograms of the aqueous layer.
A) Kinmon Daiô (grade A), B) Kinmon Daiô (grade B), C) Gaô, D) Batei Daiô. Conditions : (ii c).

and aloe-emodin 8-*O*-*β*-D-glucoside (22), and 3, 5, 4′-trihydroxystilbene 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucoside (24) and 6-hydroxymusizin 8-*O*-*β*-D-glucoside (25), are eluted almost equally, therefore the quantitative determination of these compounds by this chromatographic condition is impossible. Rhatannins appear as broad peaks at retention times, *ca* 30-50 min and 55-70 min. On the other hand, the chromatograms (Figs. 3 and 4) of the ethyl acetate-soluble portion prepared by the method (B) show clearly separated sharper peaks, except for those corresponding to (−)-epicatechin 3-*O*-*β*-D-glucoside (18), aloe-emodin 8-*O*-D-glucoside (21), lindleyin (20) and isolindleyin (22) ; the separation of these compounds can be achieved by using the solvent system (b). The aqueous layer affords relatively simple chromatographic patterns (Figs. 5 and 6), showing peaks attributable to sennosides A (16) and B (13), rhein 8-*O*-*β*-D-glucoside (14), 6-*O*-galloylglucose (2), 1,6-di-*O*-galloyl-*β*-D-glucose (8), gallic acid 3-*O*-*β*-D-(6′-*O*-galloyl)-glucoside (5) and gallic acid 4-*O*-*β*-D-(6′-*O*-galloyl)-glucoside (6). The peaks due to sennosides are particularly distinguishable from others by detection at 350 nm, while only anthraquinones can be specifically detected at the visible wavelength 420 nm.

Discussion

Although HPLC analysis using the condition (i) causes somewhat the overlap of peaks, this method may be suitable for understanding the general features of rhubarbs. On the other hand, the second chromatographic condition (ii) may be much more useful for the simultaneous determination of *μ*mol levels of almost all the components in rhubarbs. The contents of the constituents in thirty-four commercially available rhubarbs, determined by the latter method, are summarized in Table III. From this, it is evident that the contents of almost all compounds differ markedly, and also the values of standard deviation are large in any case. Fig. 7 shows the contents of biologically active compounds in Kinmon Daiô (Jing-Wuen-Da-Huang) ; 錦紋大黃, Gaô (Ija-Huang) ; 雅黃, and Batei Daiô (Mar-Tie-

Table III Contents of components in commercially available rhubarbs (34 Samples)

Compound No.	Contents (mg/g)	Average Contents (mg/g)	Standard Deviation
Sennosides			
16	0.81-13.25	5.37	3.37
13	nd- 5.16	2.24	1.46
Anthraquinones			
27	0.06-15.20	3.47	3.30
33	0.07- 4.63	1.58	1.22
34	0.06-10.04	2.16	1.44
14	0.61-14.19	7.16	4.44
21	0.13- 5.76	1.82	1.09
31	nd-18.20	2.66	2.67
30	tr- 9.60	2.35	1.89
32	nd- 3.89	1.76	0.87
Lindleyins			
20	nd- 8.91	2.12	1.92
22	nd-12.82	3.28	3.33
Stilbenes			
15	nd- 5.36	1.29	1.34
23	nd		
24	nd-20.08	4.27	5.60
Naphthalenes			
25	nd- 5.58	1.47	1.54
28	nd- 5.14	0.73	1.50
Galloylglucoses			
5	nd- 2.37	0.28	0.36
6	nd- 0.48	0.12	0.18
2	nd- 7.08	2.26	3.10
8	nd- 0.26	0.09	0.06

nd : not detected, tr : trace

Huang); 馬蹄大黄. Remarkable quantitative differences in types of rhubarbs and even in the rhubarbs with the same commercial names are easily seen from this, indicating strongly that biological and pharmacological activities differ in these rhubarbs.

Conclusion

Previous studies on the chemical evaluation of rhubarbs were focused mainly upon the quantitative determination of anthraquinones and dianthrones, especially of sennosides which are the active principles for the purgative effect. However, as mentioned above, rhubarbs possess several important biological and pharmacological activities, and their active components have recently been isolated and structurally elucidated. Under these circumstances, the exploitation of a new method for the evaluation of rhubarbs has been desired. In the present study, we have developed simple and convenient HPLC techniques for both the qualitative and quantitative determination of the ingredients, whereby it has become possible to make a simultaneous determination of μmol levels of almost all the components in rhubarbs, and hence to judge the quality of rhubarbs easily. Application of these methods to analysis of commercially availabe Chinese

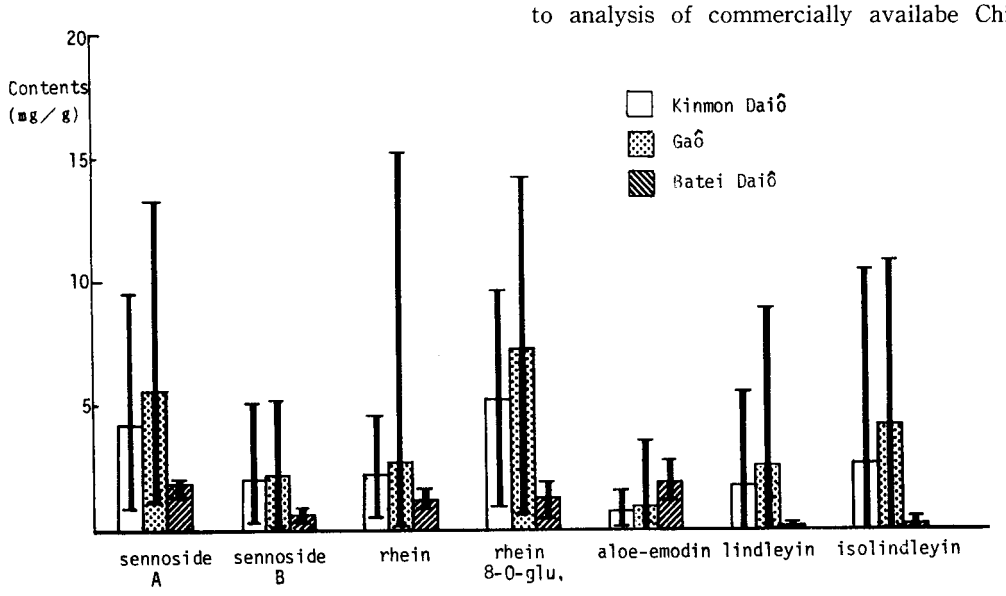

Fig. 7 Contents of biologically active components in rhubarbs.

rhubarbs showed striking differences in the composition and contents of the constituents even in those with the same commercial names. Therefore, strict evaluation of rhubarbs will be needed if they are used for medicinal purposes, and rhubarbs of high quality, especially those containing high levels of biologically active components, should be selected when intended for medical treatment.

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