# Studies on Rhubarb (Rhei Rhizoma). XV.[1] Simultaneous Determination of Phenolic Constituents by High-Performance Liquid Chromatography

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**Methods for simultaneous determination of phenolic constituents in rhubarbs have been established, making it possible to analyze almost all the phenolics, *i.e.*, anthraquinones, anthrones, phenylbutanones, stilbenes, tannins, *etc.* Application of these methods to the evaluation of commercial rhubarbs has revealed that they can be classified into several groups on the basis of their chromatographic features.**

**Keywords** rhubarb; Polygonaceae; evaluation; high-performance liquid chromatography

Rhubarbs have been employed as a purgative crude drug, and their quality has been evaluated in terms of the contents of the purgative constituents, *viz.*, sennosides. However, in Chinese traditional herbal therapy, they have been used in combination with other crude drugs for the treatments of many diseases such as a blood-stasis syndrome, hypertension, mental and renal disorders, diarrhea, urticaria, *etc.* In addition, recent phytochemical, biochemical and pharmacological examinations of the rhubarbs have led to the discovery of new biological activities and the relevant active components listed in Table I. It is therefore of great significance to develop new procedures for the simultaneous determination of the constituents. Furthermore, most rhubarbs are produced in China, especially in the western areas, and are exported under various commercial names according to their appearance, quality or place of production. Since 1970, we have been chemically investigating phenolic constituents in rhubarbs of various commercial names, and we have found that the patterns of the constituents are quite different both qualitatively and quantitatively, even among samples with the same commercial names. For these reasons, it is necessary to evaluate chemically the commercial rhubarbs. The aims of the present study were to establish procedures for the simultaneous determination of the phenolic constituents in rhubarbs by high-performance liquid chromatography (HPLC) and further to apply these methods to analysis of various rhubarbs.

## Experimental

**Materials** The rhubarbs used for HPLC analysis were mostly obtained commercially; some were gifts from Tsumura Juntendo Co., Ltd., Nihon Funmatsu Co., Ltd., and Mikuni Co., Ltd. The commercial names, as well as the places of production (or purchase), are listed in Table II. Chemical standard samples were those isolated previously from various types of rhubarbs, and are listed in Table III.

TABLE I. Biological Activities and Active Components in Rhubarbs[2]

| Activity | Active component |
|---|---|
| Psychotropic activity[3] | RG-tannin |
| Improvement of nitrogen metabolism[4] | Rhatannins (i.p.) |
| Improvement of renal discorder[5] | Tannins |
| Inhibition of angiotensin-converting enzyme (ACE)[6] | Rhatannins |
| Anti-inflammatory and analgestic activities[7] | Lindleyin |
| Purgative activity | Sennosides, rheinosides[8] |
| Anti-bacterial and anti-fungal activities | Rhein, aloe-emodin |
| Anti-tumor activity | Rhein, emodin |

**Chromatographic Conditions** The high-performance liquid chromatograph consisted of a Toyo Soda CCPM solvent delivery system, a UV-8000 spectrometer, and a Nucleosil $5C_{18}$ (M. Nagel) column (4 mm i.d. × 250 mm). The column temperature was set at 45 °C and the detector at the wavelength of 280 nm. a) Elution was performed at the flow rate of 0.8 ml/min with increasing amounts of acetonitrile in 0.05 M phosphoric acid solution (curve a in Fig. 1). Quantitative determination was made by the peak area method using a Shimadzu Chromatopak E1A. b) Elution was carried out at ambient temperature at a flow rate of 0.75 ml/min and the gradient system was like the curve b in Fig. 1. c) When the column of Cosmosil 5Ph (Nakarai Chemicals, Ltd.) (4.6 mm i.d. × 250 mm) was used, the column temperature was set at 40 °C and the gradient elution was according to the curve c (Fig. 1).

**Sample Preparation** Finely powdered rhubarb (200 mg) was extracted three times (each 24 h) with acetone–water (4:1) (each 10 ml) at room temperature. The solvent was evaporated off under reduced pressure, and the residue was treated with ethyl acetate–acetone–80% aqueous dioxane (4:3:3) (10 ml). After removal of the insolubles by filtration, the soluble portion was concentrated to dryness, and the residue was dissolved in 10 ml of ethyl acetate–acetone–dioxane (4:3:3). A portion (0.5 ml) of this solution was applied to a SEP-PAK DIOL cartridge (Waters Associates). Elution with 4 ml of ethyl acetate–acetone–dioxane (4:3:3) afforded a

TABLE II. Rhubarb Specimens Examined

| No. | Commercial name | Place of production or market |
|---|---|---|
| 1— 4 | Meng-da-huang (錦紋大黄) | Kansu (甘粛) |
| 5— 8 | Meng-da-huang (錦紋大黄) | Chinghai (青海) |
| 9—15 | Ya-huang (雅黄) | Szechwan (四川) |
| 16 | Grade II | Szechwan (四川) |
| 17 | Grade III | Szechwan (四川) |
| 18 | Dung-uai (等外) | Szechwan (四川) |
| 19 | Kung-huang (根黄) | Szechwan (四川) |
| 20, 21 | Cho-seon-dae-hwang (朝鮮大黄) | North Korea |
| 22 | *Rheum palmatum*[9] | |
| 23 | *Rheum officinale*[9] | |
| 24 | Meng-ung-da-huang (錦紋大黄) | Shanghai market |
| 25 | Chong-gi-huang (長吉黄) | Hong Kong market |
| 26—28 | Meng-ung-da-huang (錦紋大黄) | Hong Kong market |
| 29—34 | Ya-huang (雅黄) | Hong Kong market |
| 35 | Grade III | Hong Kong market |
| 36, 37 | Dung-uai (等外) | Hong Kong market |
| 38—40 | Mar-tie-da-huang (馬蹄大黄) | Hong Kong market |
| 41, 42 | Yu-da-huang (芋大黄) | Hong Kong market |
| 43 | Chwan-da-huang (川大黄) | Taipei market |
| 44 | Sang-da-huang (鮮大黄) | Osaka market |
| 45 | Hua-pe-da-huang (华北大黄) | Osaka market |
| 46 | Hokkai-daioh (北海大黄) | Hokkaido |
| 47—49 | Unknown | Tokyo market |
| 50—52 | JP rhubarb[a] | Tokyo and Osaka markets |

a) Japanese Pharmacopeial rhubarb.

TABLE III. Standard Samples

| No. | Compound |
|---|---|
| | Anthraquinones |
| 1 | Aloe-emodin[10] |
| 2 | Rhein[10] |
| 3 | Emodin[10] |
| 4 | Chrysophanol[10] |
| 5 | Aloe-emodin 8-*O*-*β*-D-glucopyranoside[10] |
| 6 | Rhein 8-*O*-*β*-D-glucopyranoside[10] |
| 7 | Chrysophanol 1-*O*-*β*-D-glucopyranoside[10] |
| 8 | Emodin 8-*O*-*β*-D-glucopyranoside[10] |
| 9 | Chrysophanol 8-*O*-*β*-D-glucopyranoside[10] |
| | Anthrones |
| 10 | Sennoside A |
| 11 | Sennoside B |
| 12 | Rheinoside A[8] |
| 13 | Rheinoside B[8] |
| 14 | Rheinoside C[8] |
| 15 | Rheinoside D[8] |
| | Phenylbutanones |
| 16 | Lindleyin[11] |
| 17 | Isolindleyin[12] |
| 18 | Isolindleyin 6″-*O*-gallate[13] |
| 19 | Isolindleyin 6″-*O*-*p*-coumaroate[13] |
| 20 | Isolindleyin 6″-*O*-cinnamate[13] |
| | Stilbenes |
| 21 | Resveratrol 4′-*O*-*β*-D-glucopyranoside[14] |
| 22 | Piceatannol 3′-*O*-*β*-D-glucopyranoside[15] |
| 23 | Rhaponticin[15] |
| 24 | Resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside[15] |
| 25 | Resveratrol 4′-*O*-*β*-D-(2″-*O*-galloyl)-glucopyranoside[13] |
| 26 | Desoxyrhaponticin[15] |
| | Naphthalenes |
| 27 | 6-Hydroxy musizin 8-*O*-*β*-D-glucopyranoside[16] |
| 28 | Torachrysone 8-*O*-*β*-D-glucopyranoside[16] |
| | Flavan-3-ols |
| 29 | (+)-Catechin 8-*C*-*β*-D-glucopyranoside[17] |
| 30 | (+)-Catechin 5-*O*-*β*-D-glucopyranoside[18] |
| 31 | (+)-Catechin[11] |
| 32 | (−)-Epicatechin 3-*O*-gallate[11] |
| | Procyanidins |
| 33 | Procyanidin B-1[19] |
| 34 | Procyanidin B-2[19] |
| 35 | Procyanidin B-1 3-*O*-gallate[11] |
| 36 | Procyanidin B-2 3′-*O*-gallate[19] |
| 37 | Procyanidin B-2 3,3′-di-*O*-gallate[11] |
| 38 | Procyanidin B-5 3,3′-di-*O*-gallate[19] |
| 39 | Procyanidin C-1 3,3′,3″-tri-*O*-gallate[19] |
| | Galloylglucoses |
| 40 | 1-*O*-Galloyl-*β*-D-glucose(*β*-glucogallin)[20] |
| 41 | 6-*O*-Galloylglucose[12] |
| 42 | 1,2-Di-*O*-galloyl-*β*-D-glucose[21] |
| 43 | 1,6-Di-*O*-galloyl-*β*-D-glucose[12] |
| 44 | Gallic acid 3-*O*-*β*-D-glucopyranoside[12] |
| 45 | Gallic acid 4-*O*-*β*-D-glucopyranoside[12] |
| 46 | 1,2,6-Tri-*O*-galloyl-*β*-D-glucose[11] |
| | Acylglucoses |
| 47 | 2-*O*-Cinnamoylglucose[20] |
| 48 | 1-*O*-Galloyl-2-*O*-cinnamoyl-*β*-D-glucose[20] |
| 49 | 1-*O*-Galloyl-6-*O*-cinnamoyl-*β*-D-glucose[21] |
| 50 | 1,6-Di-*O*-galloyl-2-*O*-cinnamoyl-*β*-D-glucose[20] |
| 51 | 1-*O*-Galloyl-2-*O*-*p*-coumaroyl-*β*-D-glucose[20] |
| | Phenol carboxylic acid |
| 52 | Gallic acid |

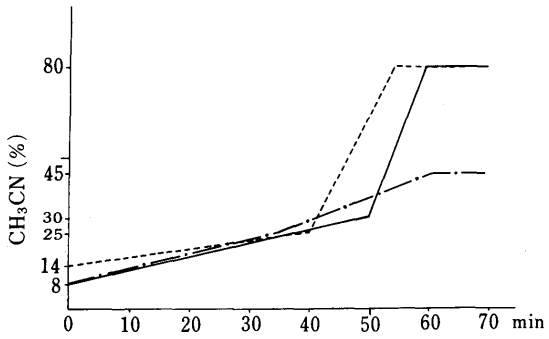


Fig. 1. HPLC Gradient Systems

a, ——; b, — · —; c, ------.

fraction of lower-molecular-weight phenolics. Subsequent elution with methanol–water (9:1) afforded a fraction containing polymeric procyanidins. On the other hand, the above ethyl acetate–acetone–80% aqueous dioxane-insoluble portion was dissolved in 10 ml of methanol–water (3:7). A portion (0.5 ml) of this solution was subjected to SEP-PAK $C_{18}$ cartridge (Waters Associates) chromatography. Elution first with 4 ml of methanol–water (3:7), and then with 4 ml of methanol afforded fractions, which were combined with the above fractions containing lower-molecular-weight phenolics and polymeric procyanidins, respectively. Each fraction was concentrated to dryness, and the residue was dissolved in 0.5 ml of methanol–water (4:1) and subjected to HPLC analysis by using conditions (a) and (c) for the lower-molecular-weight phenolic fraction and condition (b) for the polymeric procyanidin fraction.

## Results and Discussion

The chromatograms of the lower-molecular-weight phenolic fractions analyzed under condition (a) showed clearly separated peaks, the majority of which could be identified by comparisons of their $t_R$ values with those of authentic samples or by co-chromatography. Although four groups of compounds, *i.e.*, procyanidin B-1 3-*O*-gallate (**35**) and 1,2,6-tri-*O*-galloyl-*β*-D-glucose (**46**); resveratrol 4′-*O*-*β*-D-glucopyranoside (**21**), 2-*O*-cinnamoylglucose (**47**) and 1-*O*-galloyl-2-*O*-*p*-coumaroyl-*β*-D-glucose (**51**); sennoside B (**11**), resveratrol 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**24**) and resveratrol 4′-*O*-*β*-D-(2″-*O*-galloyl)-glucopyranoside (**25**); isolindleyin 6″-*O*-gallate (**18**), procyanidin B-5 3,3′-di-*O*-gallate (**38**) and 1-*O*-galloyl-2-*O*-cinnamoyl-*β*-D-glucose (**48**), were eluted at almost the same times, the separation of these compounds could be achieved by using condition (c) [Fig. 2; sample E]. The chromatograms of the polymeric procyanidin fraction showed two broad peaks at *ca.* 20—40 min and 45—55 min, which were shown to correspond to RG-tannin and rhatannins, respectively, by comparisons with authentic samples.[3,10]

The chromatograms of the lower-molecular-weight phenolic fractions in rhubarbs produced in the same districts were similar, and typical chromatograms of materials from four different origins are shown in Fig. 2 (samples A—D). The chromatograms of the polymeric procyanidin fractions in rhubarbs of different origins are shown in Fig. 3. In addition, the contents of the constituents in twenty-three rhubarbs are summarized in Table IV.

The phenolics of the rhubarbs can be classified structurally into ten groups, *i.e.*, anthraquinones, anthrones, phenylbutanones, stilbenes, naphthalenes, flavan-3-ols, procyanidins, galloylglucoses, acylglucoses and phenol carboxylic acids. Figure 4 shows the subtotal contents of each

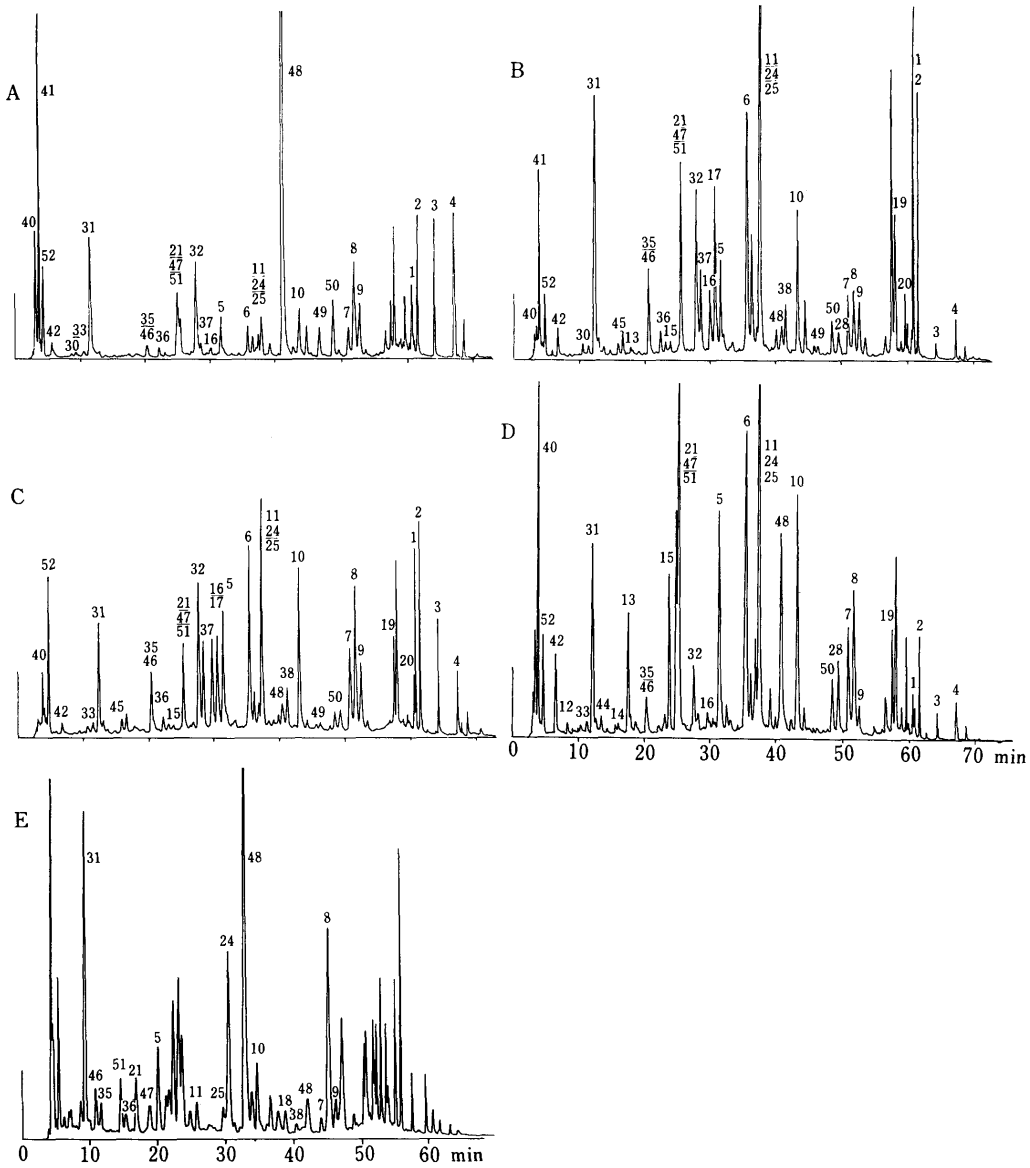


Fig. 2. High-Performance Liquid Chromatograms of Lower-Molecular-Weight Phenolic Fractions Analyzed under Conditions (a) (A—D) and (c) (E)

A, rhubarb produced in Kansu; B, rhubarb produced in Chinghai; C, rhubarb produced in Szechwan; D, rhubarb produced in North Korea; E, rhubarb of unknown origin.

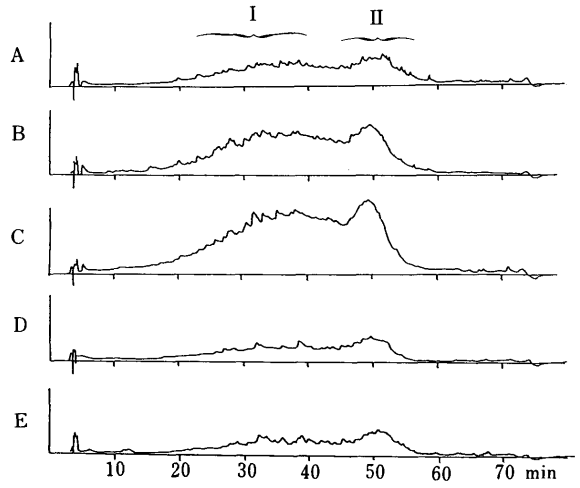


Fig. 3. High-Performance Liquid Chromatograms of Polymeric Procyanidin Fractions Analyzed under Condition (b)

A, rhubarb produced in Kansu; B, rhubarb produced in Chinghai; C, rhubarb produced in Szechwan (type I); D, rhubarb produced in Szechwan (type II); E, rhubarb produced in North Korea; I, procyanidin octamer gallates; II, procyanidin decamer gallates.

groups except for those of naphthalenes and phenol carboxylic acids. From these graphs, the following points became clear.

1. In rhubarbs produced in Kansu (甘粛), the amounts of acylglucoses were the highest among rhubarbs examined, whereas the contents of anthrones, phenylbutanones, stilbenes and polymeric procyanidins were small. These characteristics are similar to those of *Rheum palmatum*, suggesting that the Kansu rhubarbs originated from *R. palmatum*.

2. The rhubarbs produced in Chinghai (青海) contained relatively large amounts of phenylbutanones, stilbenes and polymeric procyanidins.

3. The rhubarbs produced in Szechwan (四川), which contained comparatively large amounts of anthraquinones, could be divided into two groups, types I and II, based on the levels of phenylbutanones, stilbenes and procyanidins. The contents of the polymeric procyanidins in type I are the highest, whereas those in type II are small.

4. The North Korean rhubarbs (朝鮮大黄) contained large quantities of anthrones and stilbenes. In particular, the amounts of rheinosides were much higher than those of

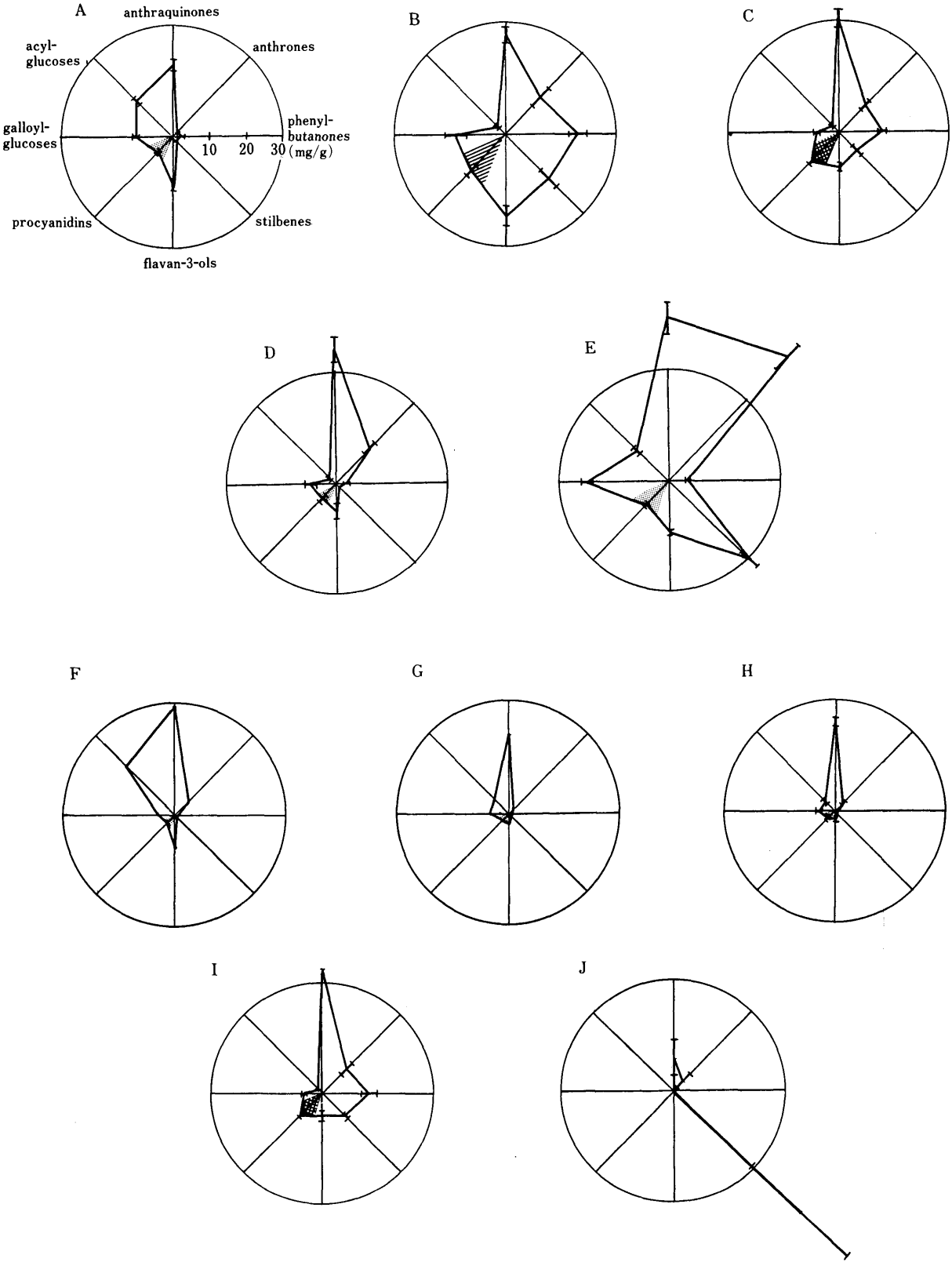


Fig. 4. The Contents of Components in Rhubarbs

A, rhubarb produced in Kansu; B, rhubarb produced in Chinghai; C, rhubarb produced in Szechwan (type I); D, rhubarb produced in Szechwan (type II); E, rhubarb produced in North Korea; F, *Rheum palmatum*; G, *Rheum officinale*; H, Mar-tie-da-huang; I, JP rhubarb; J, nonmedicinal rhubarb. The levels of polymeric procyanidins. ▦, low; ▨, relatively high; ▩, high.

sennosides, which is characteristic. On the other hand, phenylbutanone and polymeric procyanidin levels were low.

5. All of the samples of Mar-tie-da-huang (馬蹄大黄), whose origins are unknown, showed similar chromatograms. Relatively large amounts of anthraquinones and low contents of other constituents are characteristic. Since these characteristics resemble those of *R. officinale*, these rhubarbs might have originated from that species.

TABLE IV. Contents of Components in Rhubarbs

| | Place of production | | | | | | |
|---|---|---|---|---|---|---|---|
| | Kansu (甘粛) ($n=4$) | Chinghai (青海) ($n=4$) | Szechwan (I) (四川) ($n=7$) | Szechwan (II) (四川) ($n=4$) | North Korea (北朝鮮) ($n=2$) | *R. palmatum* | *R. officinale* |
| Weight of extract | 453.69±17.67 | 490.75±21.68 | 418.21±14.29 | 338.88±36.08 | 506.25±54.50 | 511.0 | 320.25 |
| Anthraquinones | | | | | | | |
| 1 Aloe-emodin | 1.75±0.06 | 0.42±0.23 | 1.33±0.40 | 0.73±0.17 | 1.64±0.87 | 1.35 | 1.01 |
| 2 Rhein | 3.08±0.24 | 4.50±0.50 | 4.27±0.60 | 1.98±0.51 | 2.76±0.90 | 2.89 | 0.50 |
| 3 Emodin | 1.49±0.13 | 0.29±0.08 | 1.98±0.38 | 1.80±0.49 | 0.80±0.30 | 6.05 | 0.88 |
| 4 Chrysophanol | 1.38±0.09 | 0.83±0.16 | 1.24±0.19 | 0.92±0.19 | 0.93±0.29 | 1.65 | 3.96 |
| 5 Aloe-emodin 8-*O*-Glc | 1.94±0.32 | 4.04±0.73 | 3.59±0.37 | 3.76±1.09 | 9.12±0.15 | 2.11 | 2.57 |
| 6 Rhein 8-*O*-Glc | 2.33±0.34 | 8.72±1.49 | 9.49±1.05 | 9.81±1.19 | 16.50±0.72 | 2.19 | 1.08 |
| 7 Chrysophanol 1-*O*-Glc | 1.63±0.51 | 2.28±0.40 | 2.22±0.24 | 3.10±0.51 | 4.26±0.02 | 1.86 | 2.31 |
| 8 Emodin 8-*O*-Glc | 3.47±0.41 | 3.40±0.32 | 5.10±0.69 | 10.10±1.29 | 6.31±0.46 | 9.56 | 2.50 |
| 9 Chrysophanol 8-*O*-Glc | 1.73±0.28 | 2.81±0.73 | 1.95±0.29 | 2.87±0.33 | 1.72±0.50 | 2.11 | 6.09 |
| Subtotal | 18.77±1.35 | 26.53±2.35 | 30.19±2.70 | 36.06±3.50 | 44.06±4.24 | 29.77 | 20.90 |
| Anthrones | | | | | | | |
| 10 Sennoside A | 1.10±0.14 | 6.73±1.29 | 6.06±0.78 | 8.34±1.30 | 12.96±0.62 | 3.30 | 0.67 |
| 11 Sennoside B | 0.76±0.18 | 3.62±0.67 | 2.82±0.44 | 3.44±0.40 | 6.85±0.22 | 1.31 | 0.38 |
| 12 Rheinoside A | n.d. | n.d. | n.d. | n.d. | 2.18±0.15 | n.d. | n.d. |
| 13 Rheinoside B | n.d. | 1.89±1.11 | 0.76±0.41 | 0.76±0.52 | 13.05±2.77 | n.d. | n.d. |
| 14 Rheinoside C | n.d. | n.d. | n.d. | n.d. | 1.84±0.22 | n.d. | n.d. |
| 15 Rheinoside D | n.d. | 1.32±0.62 | 0.44±0.25 | 0.78±0.31 | 9.56±2.06 | n.d. | n.d. |
| Subtotal | 1.86±0.32 | 13.66±3.18 | 10.08±1.67 | 13.32±1.97 | 46.46±4.48 | 4.61 | 1.05 |
| Phenylbutanones | | | | | | | |
| 16 Lindleyin | 0.94±0.13 | 3.52±0.18 | 3.72±0.33 | 0.48±0.21 | 1.32±0.12 | 0.24 | 0.12 |
| 17 Isolindleyin | 0.29±0.06 | 8.30±1.09 | 3.88±0.56 | 1.27±0.65 | 0.67±0.06 | 0.39 | 0.29 |
| 18 Isolindleyin 6″-G | n.d. | 0.89±0.33 | 0.39±0.11 | 0.27±0.17 | n.d. | n.d. | n.d. |
| 19 Isolindleyin 6″-Coum | 1.25±0.40 | 4.41±0.53 | 1.94±0.29 | 0.62±0.37 | 2.80±0.39 | n.d. | n.d. |
| 20 Isolindleyin 6″-Cinn | 0.11±0.07 | 2.56±0.98 | 1.56±0.34 | 0.07±0.07 | 0.13±0.13 | n.d. | n.d. |
| Subtotal | 2.61±0.47 | 19.67±2.54 | 11.48±1.08 | 2.71±0.57 | 4.91±0.08 | 0.73 | 0.41 |
| Stilbenes | | | | | | | |
| 21 Resveratrol 4′-*O*-Glc | 0.51±0.30 | 3.46±1.20 | 1.29±0.35 | 0.37±0.24 | 9.90±2.02 | 0.23 | 0.01 |
| 22 Piceatannol 3′-*O*-Glc | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 23 Rhaponticin | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 24 Resveratrol 4′-(6″-G)Glc | 0.60±0.23 | 10.28±1.75 | 4.58±1.32 | 0.55±0.21 | 18.43±4.93 | 0.13 | n.d. |
| 25 Resveratrol 4′-(2″-G)Glc | n.d. | 2.95±0.33 | 0.89±0.31 | n.d. | 1.87±0.04 | n.d. | n.d. |
| 26 Desoxyrhaponticin | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Subtotal | 1.11±0.53 | 16.68±2.86 | 6.57±1.95 | 0.92±0.31 | 30.19±2.95 | 0.36 | 0.01 |
| Naphthalenes | | | | | | | |
| 27 6-OH musizin 8-*O*-Glc | 1.82±0.15 | 2.02±0.40 | 0.93±0.33 | 0.68±0.68 | 6.05±0.22 | 0.42 | 0.42 |
| 28 Torachrysone 8-*O*-Glc | 0.89±0.14 | 2.45±0.23 | 1.53±0.33 | 1.25±0.53 | 6.43±0.53 | 0.54 | 1.73 |
| Subtotal | 2.76±0.23 | 4.46±0.62 | 2.46±0.47 | 1.93±1.21 | 12.48±0.32 | 0.96 | 2.15 |
| Flavan-3-ols | | | | | | | |
| 29 Catechin 8-*C*-Glc | 0.10±0.09 | 0.70±0.18 | 0.10±0.07 | n.d. | n.d. | 0.12 | 0.01 |
| 30 Catechin 5-*O*-Glc | 0.23±0.14 | 0.66±0.24 | 0.16±0.07 | 0.09±0.09 | 0.48±0.48 | n.d. | n.d. |
| 31 Catechin | 8.22±1.24 | 13.02±1.72 | 4.46±0.82 | 4.04±1.12 | 10.86±0.09 | 6.06 | 1.18 |
| 32 Epicatechin 3-G | 5.05±0.64 | 7.46±0.73 | 4.46±0.62 | 3.43±1.24 | 2.68±0.43 | 2.75 | 1.25 |
| Subtotal | 13.60±1.24 | 21.58±2.50 | 9.46±1.22 | 7.56±2.24 | 14.02±0.82 | 8.93 | 2.44 |
| Procyanidins | | | | | | | |
| 33 Procyanidin B-1 | 0.82±0.09 | 1.21±0.12 | 0.93±0.32 | 0.48±0.17 | 2.19±0.04 | 0.77 | 0.21 |
| 34 Procyanidin B-2 | 0.18±0.11 | 0.72±0.31 | 1.17±0.24 | n.d. | 0.84±0.42 | 0.65 | 0.46 |
| 35 Procyanidin B-1 3-G | 1.08±0.17 | 2.80±0.41 | 2.20±0.28 | 1.03±0.23 | 1.83±0.15 | 0.17 | 0.20 |
| 36 Procyanidin B-2 3′-G | 1.40±0.17 | 2.20±0.41 | 1.34±0.25 | 1.13±0.33 | 1.22±0.32 | 0.28 | 0.55 |
| 37 Procyanidin B-2 3,3′-di-G | 1.15±0.16 | 4.30±0.74 | 3.22±0.42 | 1.44±0.41 | 1.37±0.27 | 1.20 | 1.12 |
| 38 Procyanidin B-5 3,3′-di-G | 0.32±0.03 | 1.45±0.16 | 1.01±0.17 | 0.23±0.15 | 0.55±0.15 | n.d. | n.d. |
| 39 Procyanidin C-1 3,3′,3″tri-G | 0.25±0.25 | 0.35±0.21 | 0.96±0.11 | 0.80±0.27 | 0.63±0.63 | n.d. | n.d. |
| Subtotal | 5.20±0.60 | 13.04±1.65 | 10.43±1.17 | 5.11±1.40 | 8.63±0.34 | 3.07 | 2.54 |
| Galloylglucoses | | | | | | | |
| 40 1-G-Glc | 6.35±0.20 | 6.95±1.82 | 2.10±0.35 | 3.10±0.52 | 14.86±0.77 | 2.42 | 3.27 |
| 41 6-G-Glc | 1.80±0.49 | 1.65±0.39 | 2.04±0.21 | 1.72±0.61 | 1.12±0.46 | 0.68 | 0.62 |
| 42 1,2-Di-G-Glc | 0.72±0.11 | 1.27±0.34 | 0.32±0.12 | 0.47±0.17 | 3.02±0.03 | 0.55 | 0.41 |
| 43 1,6-Di-G-Glc | 0.43±0.34 | 0.57±0.09 | 0.46±0.05 | 0.50±0.06 | 0.80±0.09 | 0.43 | 0.28 |
| 44 GA 3-*O*-(6″-G)Glc | 0.34±0.10 | 0.88±0.63 | 0.15±0.04 | 0.04±0.04 | 0.84±0.01 | 0.34 | 0.27 |
| 45 GA 4-*O*-(6″-G)Glc | 0.07±0.07 | 0.76±0.14 | 0.38±0.09 | n.d. | 0.62±0.08 | n.d. | 0.01 |
| 46 1,2,6-Tri-G-Glc | 0.20±0.02 | 1.42±0.34 | 0.67±0.16 | 0.83±0.28 | 0.89±0.10 | 0.36 | 0.26 |
| Subtotal | 9.89±0.81 | 13.49±3.08 | 6.11±0.74 | 6.66±1.60 | 22.19±1.36 | 4.78 | 5.12 |
| Acylglucoses | | | | | | | |
| 47 2-Cinn-Glc | 1.30±0.23 | 0.11±0.07 | 0.36±0.15 | 0.35±0.21 | 0.68±0.46 | 4.08 | 0.75 |

TABLE IV. (continued)

| | | Place of production | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kansu (甘粛) ($n=4$) | Chinghai (青海) ($n=4$) | Szechwan (I) (四川) ($n=7$) | Szechwan (II) (四川) ($n=4$) | North Korea (北朝鮮) ($n=2$) | *R. palmatum* | *R. officinale* |
| 48 | 1-G-2-Cinn-Glc | 9.29±1.00 | 0.92±0.24 | 0.94±0.25 | 0.42±0.07 | 4.18±0.62 | 9.82 | 3.54 |
| 49 | 1-G-6-Cinn-Glc | 0.58±0.07 | 0.38±0.09 | 0.19±0.06 | 0.23±0.14 | 0.21±0.04 | 0.85 | 0.31 |
| 50 | 1,6-Di-G-2-Cinn-Glc | 1.36±0.16 | 0.71±0.06 | 0.45±0.19 | 0.68±0.38 | 1.62±0.37 | 2.71 | 0.77 |
| 51 | 1-G-2-Coum-Glc | 0.86±0.08 | 0.10±0.06 | 0.19±0.08 | 0.06±0.04 | 4.93±1.54 | 1.11 | 0.54 |
| | Subtotal | 13.38±1.38 | 2.36±0.06 | 2.07±0.63 | 1.73±0.58 | 11.61±1.37 | 18.57 | 5.92 |
| Phenol carboxylic acids | | | | | | | | |
| 52 | Gallic acid | 1.68±0.32 | 0.81±0.17 | 2.37±0.45 | 1.56±0.51 | 1.92±0.18 | 1.53 | 1.28 |

Mean±S.E. mg/g. n.d., not detected.

6. The Japanese Pharmacopeial (JP) rhubarbs showed chromatograms similar to those of rhubarbs produced in Szechwan (四川) (type II).

7. The chromatographic features of Hokkai-daioh (北海大黄) cultivated in Japan were similar to those of rhubarbs produced in Chinghai (青海). This fact is in good agreement with the historical background that the seed of Hokkai-daioh was brought to Japan from Chinghai *via* a botanical garden in Berlin. This type of rhubarb is regarded as *R. tanguticum*.

8. Rhubarbs which are not used medicinally were shown to contain the largest amounts of stilbenes, *viz.* rhaponticin, desoxyrhaponticin, *etc.*

As mentioned in the introduction, rhubarbs have been employed medicinally for many purposes from ancient times. However, the qualities of commercial rhubarbs are not constant, since there are a variety of species distributed over vast areas of Asia, and their origins are almost entirely unknown. The relatively simple chromatographic procedures developed in this study will be extremely helpful for the evaluation of rhubarbs and also for getting information about the content of each component. Since rhubarbs have recently been found to possess diverse biological activities, strict selection of high-quality rhubarbs containing large amounts of pharmacological active compounds would be necessary if they are to be used clinically.

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