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Quantitative Analysis of the Laxative Components in Rhubarb by High Performance Liquid Chromatography

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A quantitative analysis of the laxative components in rhubarb was carried out by high performance liquid chromatography (HPLC).

A 70% tetrahydrofuran extract of rhubarb was heated with dilute sulfuric acid at 100°C to hydrolyze the active glycosides, sennoside and glycosyloxyanthraquinone, to the corresponding aglycones, sennidin and oxyanthraquinone. The hydrolyzate was applied to a Sep-pak C_{18} cartridge and washed with water. The aglycones retained on the cartridge were then eluted with tetrahydrofuran. The eluate was quantitatively analyzed by HPLC.

The sennoside content in the rhubarb correlated highly with the laxative activity, whereas the correlation between oxyanthraquinone content and laxative activity was rather low. Consequently, it was confirmed that, with great reliability, the laxative activity of a rhubarb can be calculated by an equation based on sennoside content.

Keywords——rhubarb; sennoside; oxyanthraquinone; high performance liquid chromatography; laxative activity; active component-activity correlation

The laxative components in rhubarb, the rhizome of *Rheum* spp. (Polygonaceae), comprise the oxyanthraquinones, their glycosides, and the dianthrone glycosides.

In a previous paper,[1] we reported a quantitative analysis of the laxative components in rhubarb by photodensitometry and suggested that the activity of the crude drug correlated highly with the sennoside content. Recently, many reports have been published on analytical studies of sennoside by high performance liquid chromatography (HPLC).[2] However, these studies involved merely either quantitative analyses of the major sennoside alone or identification of the components in a crude drug.

In this paper, a simple quantitative analysis of the active components known hitherto by HPLC is described and the correlation between the content of the active components and laxative activity[3] is discussed.

Experimental

Materials——Rhubarbs were obtained from markets in Japan, Hong Kong, Thailand, and Germany, whereas Shin-Shu Daio (a hybrid between *R. coreanum* and *R. palmatum*)[4] has been cultivated for 4 or 5 years in Hokkaido Pref., Japan.

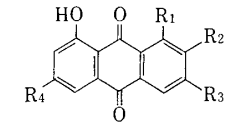
Ground rhubarb (*ca.* 50 mesh) was dried over silica gel for more than two weeks at room temperature prior to analysis and biological estimation.[5]

Biological Estimation——Laxative activity (ED_{50}: mg/kg in mice) was examined by the method of Tsukui *et al.*[5]

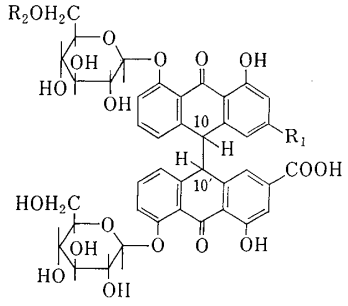
HPLC Apparatus and Condition——Pump: SHIMADZU LC-5A (Shimadzu). Detector: HITACHI variable wave length UV monitor 638-41 (Hitachi). Recorder: SHIMADZU chromatopac C-R2A(X) (Shimadzu). Column: Wakogel column ODS-10K (4ID×300 mm) (Wako). Mobile phase: CH_3CN-3% aq. CH_3COOH (46:54). Flow rate: 2 ml/min. Wave length: 270 nm.

Analytical Procedure——About 50 mg of material was weighed accurately and extracted with 15 ml of 70% THF under ultrasonication for 30 sec, and the suspension was allowed to stand at room temperature for 10 min.

TABLE I. Structure of Oxyanthraquinone and Sennoside in Rhubarb



	R_1	R_2	R_3	R_4
Chrysophanol	OH	H	CH_3	H
Physcion	OH	H	CH_3	OCH_3
Emodin	OH	H	CH_3	OH
Aloe-emodin	OH	H	CH_2OH	H
Citreorosein	OH	H	CH_2OH	OH
Rhein	OH	H	COOH	H
Laccaic acid D	CH_3	COOH	OH	OH



	R_1	R_2	10-10′
Sennoside A	COOH	H	*threo*
B	COOH	H	*erythro*
C	CH_2OH	H	*threo*
D	CH_2OH	H	*erythro*
E	COOH	OC-COOH	*threo*
F	COOH	OC-COOH	*erythro*

TABLE II. Laxative Activity of Sennoside and Oxyanthraquinone in Mice

Compounds	ED_{50} (mg/kg)	Compounds	ED_{50} (mg/kg)
Sennoside A	13.5	Aloe-emodin	59.6
Sennoside B	13.9	8-Glu. aloe-emodin	71.6
Sennoside C	13.3	Rhein	97.5
Sennoside D	15.8	8-Glu. rhein	103.0
Sennoside E	13.5	Chrysophanol	>500
Sennoside F	16.1	Physcion	>500
		Emodin	>500

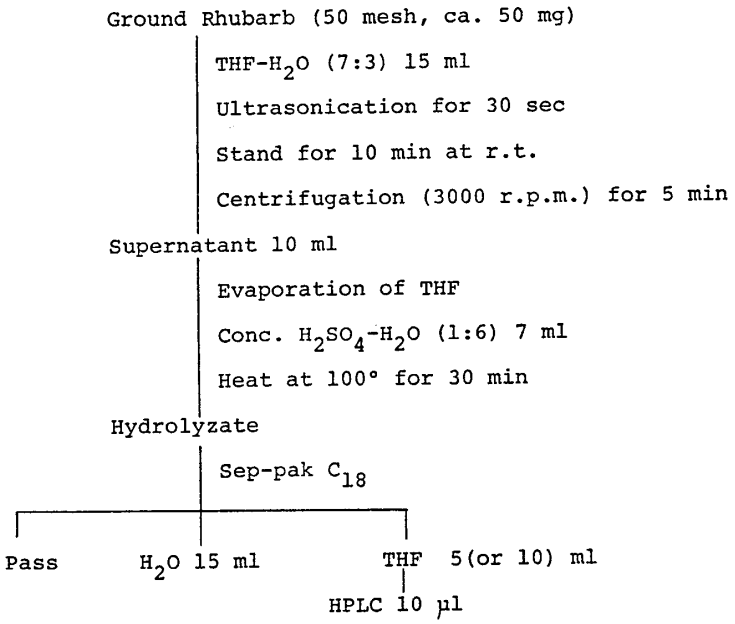


Chart. Pretreatment of Rhubarb for HPLC Analysis

After centrifugation (3000 rpm) for 5 min, 10 ml of the supernatant was taken out and THF was removed *in vacuo*. Six ml of H_2O and 1 ml of conc. H_2SO_4 were added to the aqueous solution and the solution was heated in a boiling water bath for 30 min without a reflux condenser. The reaction mixture was applied to a Sep-pak C_{18} cartridge

(Waters) and *ca.* 15 ml of H_2O was passed through the cartridge until the eluate showed neutral. The aglycones were then eluted with *ca.* 4 ml of THF; *ca.* 9 ml of THF was used as the eluent for a material more active than ED_{50} 100 mg/kg. After the eluate was diluted accurately to 5 ml (or 10 ml) with THF, 10 μl of it was applied to HPLC.

The first peak (t_R: *ca.* 7.7 min) on the chromatogram was used to determine the amount of rhein (and aloe-emodin) with the following calibration equation.

$$y=2.207\times10^{-5}x+0.04496 \qquad (0.093<y<2.33)$$

Where x and y represent the peak area ($\mu V\times sec$) and the amount of rhein (and aloe-emodin) (μg), respectively.

The second (t_R: *ca.* 11.4 min) and the third (t_R: *ca.* 14.4 min) peaks served to determine the respective amount of sennidins A and B with the following calibration equations.

for sennidin A,

$$z_1=2.915\times10^{-5}x+0.04816 \qquad (0.10<z_1<3.39)$$

for sennidin B,

$$z_2=3.141\times10^{-5}x+0.01325 \qquad (0.094<z_2<3.10)$$

Where z_1 and z_2 indicate the amount of sennidins A and B. The content of active components in a material was then calculated as follows:

$$R_O(\%)=y\times(1000E/10)\times(15/10)\div1000M\times100=15\ Ey/M$$

$$S_A(\%)=(z_1+z_2)\times(1000E/10)\times(15/10)\div1000M\times(862.75/538.47)\times100=24.033\ E(z_1+z_2)/M$$

Here R_O and S_A stand for the content of rhein (and aloe-emodin) and sennoside, respectively. M and E represent the weight of material (mg) and the volume of eluate (ml) on pretreatment, respectively. The figures of 862.75 and 538.47 correspond to the molecular weight of sennoside A (B) and sennidin A (B).

Results and Discussion

1. Analytical method

A chromatographic profile of rhubarb extract is so complex because of the six sennosides and many oxyanthraquinone derivatives that it is practically impossible, though desirable, to carry out a quantitative analysis of all active components in their intact forms. The glycosides, therefore, were hydrolyzed to the aglycones to make the chromatogram simpler. This procedure is based on the knowledge that all six sennosides have comparable activity and that the activities of both rhein and aloe-emodin are also comparable to those of their corresponding glycosides.

The retention times (t_R) and the profile of the aglycones on HPLC are shown in TABLE III and Fig. 1.

The four aglycones of the six sennosides, called sennidins, were included in two peaks: one consisted of sennidins A and C, and the other of sennidins B and D. The total amount of sennidin could be reasonably calculated using both peaks because all sennidin exhibited the same absorbance at 270 nm. The sennoside content was then determined as that of sennosides A and B because of the approximation of the molecular weight. Moreover, the sennoside content was expressed as that of sennoside A after adding the values for sennosides A and B because of the mutual isomerization of their aglycones during the procedure as described below.

Both rhein and aloe-emodin gave a single peak on the chromatogram, and this could be practically considered as that of rhein alone, because the content of rhein is much greater than that of aloe-emodin in rhubarb and the two compounds have comparable laxative activity and absorbance at 270 nm. The

TABLE III. Retention Times of The Aglycone on HPLC

Glycoside	Aglycone	t_R (min)	Glycoside	Aglycone	t_R (min)
Sennoside A	Sennidin A	11.45	8-Glu.rhein	Rhein	7.88
Sennoside E	〃	〃	8-Glu.aloe-emodin	Aloe-emodin	7.56
Sennoside B	Sennidin B	14.47		Emodin	18.95
Sennoside F	〃	〃		Chrysophanol	40.2
Sennoside C	Sennidin C	11.43		Physcion	69.6
Sennoside D	Sennidin D	14.43			

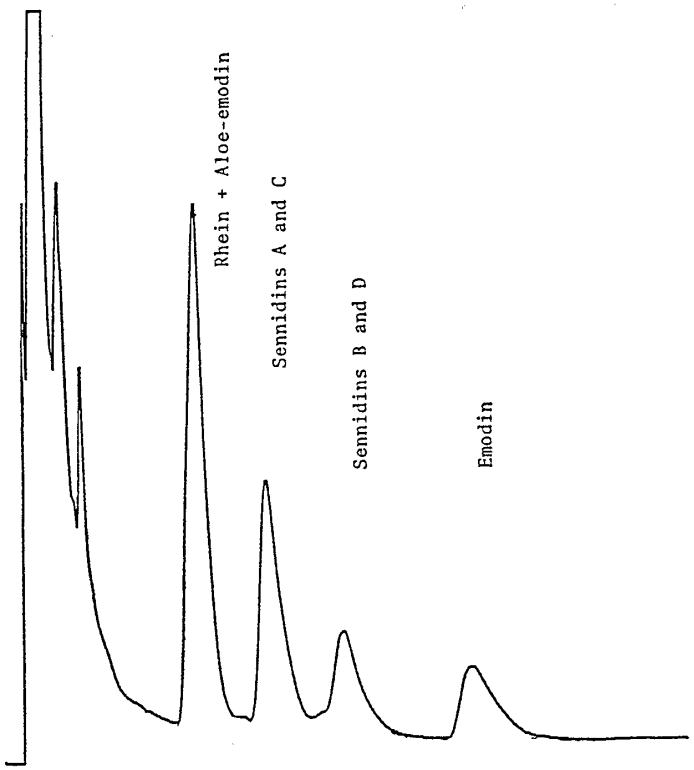


Fig. 1. HPLC Profile of Hydrolyzate

TABLE IV. Effect of Extracting Period on Yield of Active Components

Compound	Time (min) 10	20	30	40
Sennidin A	100[a)]	98.4	97.0	98.5
Sennidin B	100[a)]	99.2	92.9	92.5
Rhein	100[a)]	95.7	92.2	94.7

[a)] Yield of each component extracted for 10 min was defined as 100.

TABLE V. Isomerization Rate between Sennidin A and Sennidin B in THF Solution

Compound	Time (hr) 0	1	2	3	4	5	6	7	8	9	24
Sennidin A	100	98	96	—	90	—	82	—	74	73	59
↓											
Sennidin B	0	2	4	—	10	—	18	—	26	27	41
Sennidin B	100	97	96	93	90	88	85	83	82	79	—
↓											
Sennidin A	0	3	4	7	10	12	15	17	18	21	—

TABLE VI. Experimental Error for Quantitative Analysis of Active Components

Experiment	Sample wt. (mg)	*So* A (%)	*So* B (%)	T.*So* (%)	T.*S*A (%)	*Ro* (%)
1	51.67	0.4601	0.2344	0.6945	1.11	1.62
2	54.28	0.4647	0.2243	0.6890	1.10	1.60
3	50.19	0.5224	0.2458	0.7682	1.23	1.67
4	53.45	0.4934	0.2221	0.7155	1.15	1.55
5	49.90	0.5174	0.2444	0.7618	1.22	1.64
6	51.70	0.4985	0.2475	0.7460	1.20	1.66
Ave.		0.4928	0.2364	0.7291	1.17	1.62
S.D.					0.056	0.044
C.V.					4.84	2.72

So A: Sennidin A, *So* B: Sennidin B, T.*So*: Total Sennidin, T.*S*A: Total Sennoside, *Ro*: Rhein.

TABLE VII. Recovery Rate of Active Components

Experiment	Total Sennoside: T.*S*A Init. (mg)	Add. (mg)	T.*S*A (Calcd.) (mg)	T.*S*A (Found) (mg)	Recovery (%)	Rhein: *R*o Init. (mg)	Add. (mg)	*R*o (Calcd.) (mg)	*R*o (Found) (mg)	Recovery (%)
1	0.5979	0.9620	1.5599	1.5178	97.30	0.8278	0.8456	1.6734	1.7011	101.66
2	0.6107	0.9620	1.5727	1.5181	96.53	0.8456	0.8456	1.6912	1.6748	99.03
3	0.5868	0.9620	1.5488	1.5621	100.86	0.8124	0.8456	1.6580	1.6464	99.30
Ave.					98.23					100.00

TABLE VIII. Content of Active Components and Activity

No.	Sample	ED_{50}	$1/ED_{50}\times10^3$	*S*A (%)[a]	*R*o (%)[b]	*S*T (%)[c]
1	Germany	388	2.58	0.58	0.94	0.71
2	Batei Daio	379	2.64	0.41	0.81	0.52
3	Kinmon Daio	338	2.96	0.57	0.99	0.71
4	Thailand	290	3.45	0.85	1.54	1.07
5	Germany	269	3.72	1.12	2.45	1.46
6	Kinmon Daio	222	4.50	1.15	1.57	1.37
7	Gao	170	5.88	1.46	2.01	1.74
8	S.B[d]	263	3.80	1.17	1.62	1.40
9	S.SI-455	207	4.83	1.47	1.94	1.74
10	S.HIA-491-1	196	5.10	1.32	1.74	1.56
11	S.HF-444	171	5.85	1.46	1.93	1.73
12	S.EI6-33	76	13.16	3.79	2.16	4.09
13	S.EI2-9	73	13.70	4.14	2.87	4.54
14	S.F-272	202	4.95	1.43	1.57	1.65
15	S.F-260	190	5.26	1.71	1.76	1.96
16	S.F-288	187	5.35	1.69	1.65	1.92
17	S.F-277	186	5.38	1.50	1.81	1.75
18	S.F-271	185	5.41	1.63	1.61	1.86
19	S.F-290	174	5.75	1.77	1.61	2.00
20	S.F-263	169	5.92	1.70	1.78	1.95
21	S.F-289	162	6.17	1.95	1.73	2.19
22	S.F-276	154	6.49	1.74	2.07	2.03
23	S.F-286	138	7.25	2.05	1.84	2.31
24	S.K-7	192	5.21	1.48	1.52	1.69
25	S.K-5	132	7.58	2.13	1.76	2.38
26	S.K-4	121	8.26	2.72	1.88	2.98
27	S.K-3	113	8.85	2.39	1.67	2.62
28	S.K-8	109	9.17	2.97	1.88	3.23
29	S.K-10	105	9.52	2.06	1.67	2.29
30	S.K-11	105	9.52	2.15	1.49	2.36
31	S.K-12	96	10.42	3.25	2.26	3.57
32	S.K-1	92	10.87	3.58	2.46	3.92

[a] Sennoside (%), $n=3$.
[b] Rhein (%), $n=3$.
[c] *S*A (%)+0.14 *R*o (%).
[d] S.: Shin-Shu Daio.

content of oxyanthraquinones (*R*o), therefore, was determined roughly as that of rhein.

A quantitative analysis of emodin, chrysophanol, and physcion was omitted because of their extremely low activities. Both citreorosein and laccaic acid D were also omitted for the poor content.

2. Extraction

Following the procedure of our previous report,[1] aqueous tetrahydrofuran was chosen as the solvent for extraction.

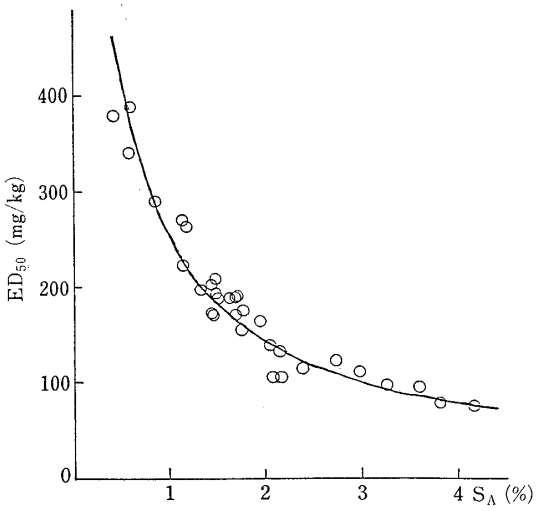


Fig. 2. Correlation between Activity (ED_{50}) and Sennoside Content (S_A)

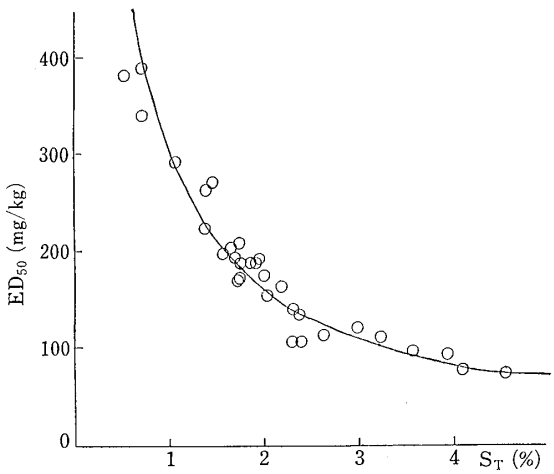


Fig. 3. Correlation between Activity (ED_{50}) and Corrected Sennoside Content (S_T)

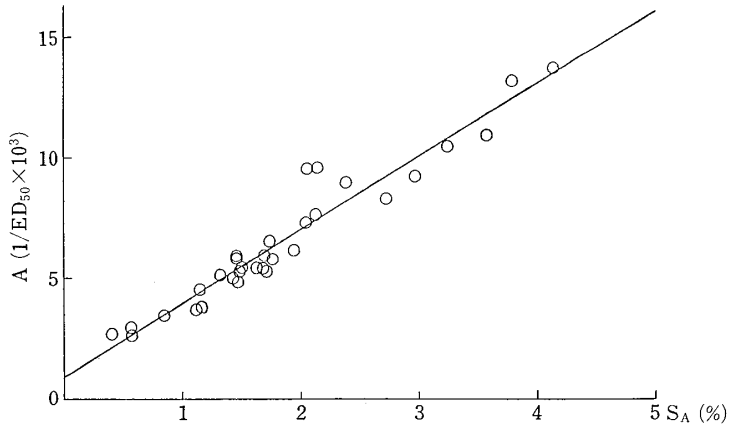


Fig. 4. Regression Curve for Correlation between Activity (A) and Sennoside Content (S_A)

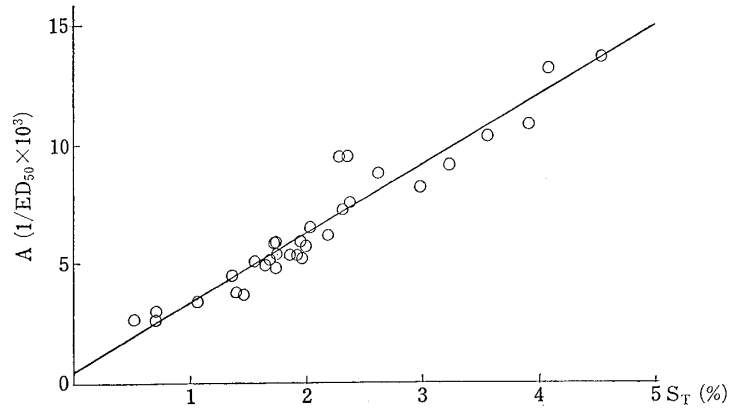


Fig. 5. Regression Curve for Correlation between Activity (A) and Corrected Sennoside Content (S_T)

TABLE IX. Observed and Calculated Laxative Activities of Rhubarb (ED_{50}: mg/kg)

No.	Observed	Calcd. (eq.2)	Calcd. (eq.3)	No.	Observed	Calcd. (eq.2)	Calcd. (eq.3)
1	388	373	395	17	186	183	181
2	379	462	505[a)]	18	185	171	171
3	338	378	395	19	174	159	160
4	290	285	280	20	169	165	164
5	269	232	213[a)]	21	162	146	147
6	222	227	226	22	154	161	158
7	170	187	182	23	138	140	140
8	263	224	221	24	192	185	187
9	207	186	182	25	132	136	136
10	196	203	201	26	121	109	110
11	171	187	183	27	113	122	124
12	76	81	81	28	109	101	102
13	73	74	74	29	105	140[a)]	141[a)]
14	202	190	191	30	105	134[a)]	137[a)]
15	190	164	163	31	96	93	93
16	187	165	166	32	92	85	85

[a)] $p>0.1$.

The duration of extraction was determined as 10 min based on the experimental results shown in TABLE IV.

3. Mutual isomerization between Sennidins A and B

It was observed that sennidins A and B isomerized mutually in THF (TABLE V).

4. Experimental error

The analysis procedure was repeated with a sample (No. 8 in TABLE VIII) in order to estimate the experimental error. The procedure appears to be reliable (TABLE VI).

5. Recovery

After sennosides A and B, and 8-glucosylrhein were added to a sample (No. 8), analysis was carried out as described above: 98.2% of the sennoside and 100% of the 8-glucosylrhein were recovered (TABLE VII).

6. Correlation between active component contents and activities

The results of the quantitative analysis and the laxative activity assay for 32 samples are shown in TABLE VIII.

Although it was presumed that the contribuion of rhein (and aloe-emodin) to the activity of a rhubarb was rather low, its content (R_O) was added to S_A after being rectified to that of the sennoside using the following equation.

$$S_T = S_A + R_O \times (13.5/97.5) = S_A + 0.14\ R_O$$

Where S_T represents the corrected amount of sennoside. The figures of 13.5 and 97.5 correspond to the ED_{50} values of sennoside A and rhein, respectively.

The activity (ED_{50}) appeared to be in hyperbolic relation with the content of the active components (Figs. 2 and 3) and the correlation equation between R_O, S_A or S_T and the activity defined by the least square method confirmed this.

$$A = 4.553\ R_O - 1.505,\ r = 0.6633 \quad (p<0.01) \tag{1}$$

$$A = 3.032\ S_A + 0.920,\ r = 0.9644 \quad (p<0.01) \tag{2}$$

$$A = 2.883\ S_T + 0.483,\ r = 0.9607 \quad (p<0.01) \tag{3}$$

Where A means $1/ED_{50} \times 10^3$.

The correlation coefficient for R_O was not so high as that for S_A. Even if S_T was chosen instead of S_A, the correlation coefficient did not increase. It is, therefore, suggested that sennoside is the main active component of rhubarb and that both rhein and aloe-emodin contribute little to the activity.

The ED_{50} values calculated from the equations (2) and (3) were in good agreement with the observed ones (TABLE IX).

Consequently, it was confirmed that an equation based on sennoside content alone can be used with great reliability to estimate the laxative activity of rhubarb.

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References and Notes

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