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Original Research Article

Evaluation of the Amounts of Sennosides A and B in Rhubarb-containing Kampo Medicines to Create a Ranking of Kampo Medicines for Appropriate Selection of Laxatives

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Abstract

Objectives: To evaluate 20 Kampo medicines, which comprised 6 formulas, Otsujito, Junchoto, Tokakujokito, Bofutsushosan, Mashiningan, and Keishikashakuyakudaioto, from 7 brands, to create a ranking of Kampo medicines for appropriate selection of laxatives.
Methods: The amounts of sennosides A and B, the important components showing laxative effects contained in Kampo medicines, were analysed using High Performance Liquid Chromatography.
Results: We found that the amounts of sennosides A and B were different among brands, even when they had the same formula. Furthermore, the amounts of sennosides differed when the same amounts of rhubarb were used.
Conclusions: These results suggest that the differences in amounts of sennosides are caused by the quality of the rhubarb used. Kampo medicines containing laxatives other than rhubarb, including disodium sulphate and hemp seed, had synergistic laxative effects. Thus, in the future, it may be possible to adjust laxative potency of Kampo medicines through further clinical tests.

Keywords

Kampo Medicines, Strength of laxative effects, Rhubarb, Sennosides A and B

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Introduction

Rhubarb (Da Huang) is an important herbal medicine used in several Kampo medicines and has laxative, stomachic, and antidiarrhoeal effects[1]. Some Rheum species (Polygonaceae), such as *Rheum palmatum* L., *R. tanguticum* Maximowicz, *R. officinale* Baillon, and *R. coreanum* Nakai, and interspecific hybrids such as Shinshu Daio (*R. coreanum* Nakai × *R. palmatum* L.) are listed as 'medicinal rhubarb' in the *Japanese Pharmacopoeia 17th edition* (JP)[2]. These rhubarb species contain not only anthraquinone and anthrone analogues such as sennosides A-F but also phenolic compounds such as tannins and stilbene derivatives[3].

Sennosides A and B, which are the active compounds of Senna leaves, are also potent components of rhubarb. Orally administered sennosides A and B are hydrolysed and converted into aglycons, forming sennidins A and B and glucose[4], by β-D-glucosidase originating from the gastrointestinal microbiota[4] (Figure 1).

The mechanisms of rheinanthrone's laxative effects are not clear, but some researchers have reported that the action of rheinanthrone stimulates Auerbach's plexus to promote colonic peristalsis, thereby inducing laxative effects[5]. Other researchers have suggested that prostaglandins and calcium ions might have roles in the laxative effects of rheinanthrone[6,7], but others have reported that rheinan-

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throne and a metabolite of sennosides C and D, namely aloe-emodinanthrone, synergistically decrease water absorption from the colon[8].

Administration of rhubarb should be avoided for anyone who is pregnant or can become pregnant because rhubarb is considered to have oxytocic action and cause hyperaemia not only in the intestines but also in uterus, which might cause early abortion[1]. Repeated administration of herbal medicines containing anthraquinone analogues, including rhubarb, senna folium, and aloe, can lead to an adverse effect called melanosis coli, in which the colonic mucosa is dyed yellowish brown to dark brown by the pigment lipofuscin[9].

The dyeing process impairs mucosal function and weakens peristalsis. This means that repeated administration of anthraquinone analogues can lead to resistance to laxation; thus, its long-term administration should be avoided[9]. The laxative effects of these medicines differ among patients, especially physically weak patients prone to developing abdominalgia and diarrhoea; therefore, physicians should use caution when prescribing laxative medicines[1,10].

For appropriate use of laxative agents, patients are first directed to change their lifestyle and administer osmotic laxatives. If these measures do not prove sufficient to improve constipation, stimulant laxatives such as sennosides may be administered[11].

Stimulant laxatives should be administered for only a short time or as a single dose. Long-term use of stimulant laxatives can lead to tolerance. Therefore, to avoid long-term use of stimulant laxatives, stronger medicines should be replaced with weaker ones based on the strength of the laxative effect and colonic transit time.

However, the strength of clinically administered stimulant laxatives remains unclear. Our previous study revealed the strength of some stimulant laxatives[12], but the data were insufficient to quantify the strength of stimulant laxatives in Kampo medicines.

Furthermore, the Research Society for the Diagnosis and Treatment of Chronic Constipation has suggested that Kampo medicines can improve chronic constipation; however, there is little scientific evidence to support clinical use[13]. Accordingly, strengths of stimulant laxatives and formulas and quality of Kampo medicines remain unclear.

In this study, the amounts of sennosides A and B in Kampo medicines containing rhubarb were analysed using high-performance liquid chromatography (HPLC) to facilitate the selection of appropriate stimulant laxatives based on their strengths.

Methods

Materials

Sennoside A (>99%) and B (>98%) and sodium hydrogen carbonate (>99%) were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Acetonitrile, distilled water, and acetic acid for the HPLC mobile phases were purchased from Nacalai Tesque (Kyoto, Japan). The following Kampo medicine dry extracts were obtained through Okazaki Surgery, Clinic for Gastroenterology and Proctology.

Seven Otsujito (Yi Zi-Tang: 03) formulas were obtained from Tsumura & Co. (Tsumura; Tokyo, Japan), Kotaro Pharmaceutical Co., Ltd. (Kotaro; Osaka, Japan), Kracie Pharmaceutical (Kracie; Tokyo, Japan), Ohsugi Pharmaceutical Co., Ltd. (Ohsugi and Junko; Osaka, Japan), Teikoku Kampo Seiyaku Co., Ltd. (Teikoku; Kagawa, Japan), and Taikoseido Pharmaceutical Co., Ltd. (Taikodo; Kobe, Japan). Two Junchoto (Run Chang-Tang: 51) formulas were obtained from Tsumura and Taikodo. Six Tokakujokito (Tao He Cheng Qi-Tang: 61) formulas were obtained from Tsumura, Kotaro, Kracie, Ohsugi, Junko, and Teikoku.

A Bofutsushosan (Fang Feng Tong Sheng-San: 62) formula was obtained from Tsumura. Three Mashiningan (Ma Zi Ren-Wan: 126) formulas were obtained from Tsumura, Kotaro, and Ohsugi.

A Keishikashakuyakudaioto (Gui Zhi Jia Shao Yao Da Huang-Tang: 134) formula was obtained from Tsumura.

Kampo formula names are denoted as ‘company name-formula number’ (e.g. Tsumura’s Otsujito is “Tsumura-03”). When formulas are referred to without a company name, the formula names are expressed in accordance with guidelines for romanising Kampo medicines’ names[14].

Preparation of standard solutions and samples

Samples of each Kampo medicine were prepared as follows. One hundred milligrammes of a Kampo medicine extract granule or fine grain was weighed and suspended in 10 mL of 1 mg/mL sodium hydrogen carbonate solution. The suspensions were sonicated for 30 min and centrifuged at 1,200 rpm for 15 min, after which the supernatants were used for HPLC analyses.

Quantitative analyses of sennosides A and B using HPLC

Sennosides A and B in Kampo medicines containing rhubarb were analysed quantitatively by HPLC. The samples and standard solutions were analysed using LC-8020 model III (Tosoh Co. Ltd., Tokyo, Japan) equipped with a TSKgel ODS-100Z silica gel column (3 μm, 4.6 mm I.D. × 250 mm; Tosoh Co. Ltd.) and a UV-8020 ultraviolet visible absorbance (UV/Vis) detector (Tosoh Co. Ltd.). These quantitative analyses were conducted as in our previous study[12].

The mobile phase was isocratic acetonitrile/1.25% acetic acid aqueous solution (1:4); the flow rate was 0.5 mL/min; and the detection wavelength was 340 nm. An analysis of the standard solutions indicated that sennosides A and B ap-

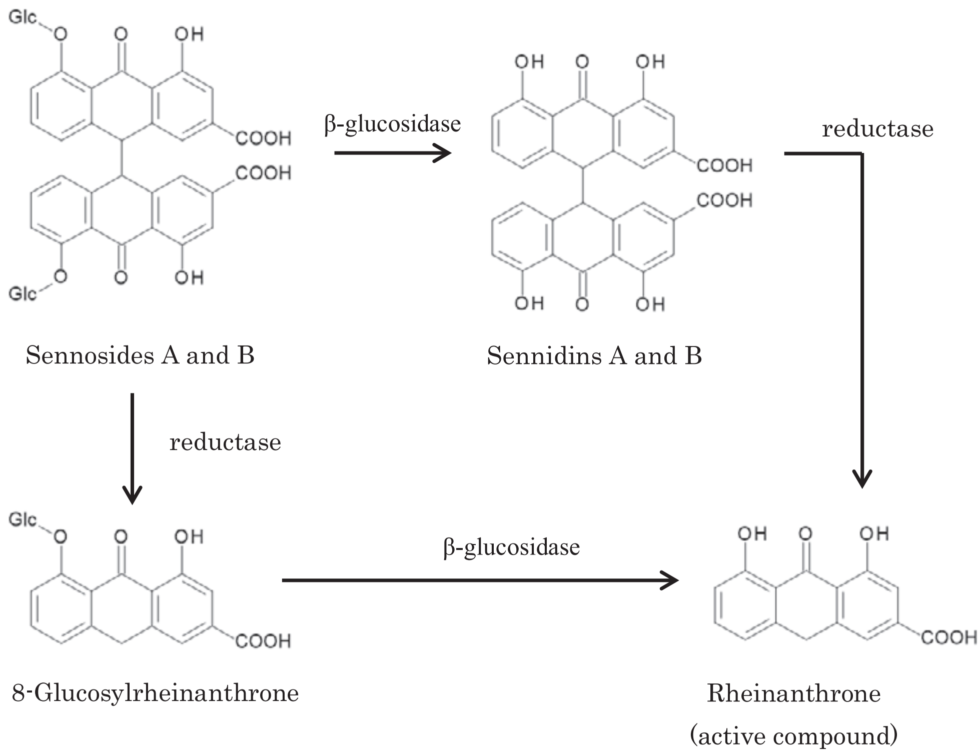

Figure 1. Main metabolic pathway of sennosides by human gastrointestinal microbiota.

peared at retention times of 23 and 13 min, respectively.

Measuring the pH of Kampo medicines in aqueous solutions

Aqueous solutions were obtained by following the same method used to prepare the HPLC samples; the Kampo medicine extract granules and fine grains were suspended in distilled water and sonicated. The sonicated suspensions were centrifuged at 4,000 rpm for 15 min, and the obtained supernatants were filtered using HLC-Disk 25 (Kanto Chemical Co., Inc, Tokyo). The pH of the filtered solutions was measured using a pH meter LAQUAact D-74 (Horiba Scientific, Kyoto, Japan).

Statistical analyses

The results are presented as mean ± standard error of the mean (SEM). Analyses of three groups were performed using Dunnett's test in GraphPad InStat (GraphPad Software Inc., San Diego, CA), and all others were performed using Student's *t*-test in Microsoft Excel 2019 (Microsoft Inc., Redmond, WA). Correlation ratios between data were calculated using Microsoft Excel.

Results

Standard curves for sennosides A and B are as presented in Figure 2. The standard curve of sennoside A indicated high linearity ($R^2 > 0.998$), and the linear range of quantification was 2.4×10^{-4} to 1 mg/mL (Figure 2A). The standard curve of sennoside B indicated high linearity ($R^2 > 0.999$), with a linear range of quantification of 4.9×10^{-4} to 1.0 mg/mL (Figure 2B).

Assigning a score of 10 to the commonly prescribed Pursennid® Tablet (Sun Pharma Japan Ltd, Tokyo, Japan), which contains 12 mg of sennosides A and B, the relative scores for the amounts of sennosides A and B in the Kampo medicines analysed using HPLC were calculated. The resulting scores indicated the relative strength of medicinal rhubarb-containing Kampo medicines (Table 1). The formula for calculating the relative score is [Amount of sennosides A and B (mg/mL)] × 10 mL × [formulas' package weight (g)] × 10 (a.u.)/[0.1 g × 12 mg].

The weakest formula was that of Tsumura-03, with a score of 1.1, and the strongest was Kracie-61, with a score of 9.7. No Kampo medicines were superior to Pursennid; however, the laxative effect of Kracie-61 was equivalent to that of Pursennid.

The pH of Otujito aqueous solutions was 4.94-5.52. The median pH was equal to that of Ohsugi-03, and the pH of each sample solution was thus statistically compared with that of Ohsugi-03 using Dunnett's test. The pH values of Kotaro-03 and Kracie-03 were significantly higher than that of Ohsugi-03, and that of Taikodo-03 was significantly lower than that of Ohsugi-03 (Table 2).

The pH of Tokakujokito aqueous solutions was 5.31-5.69. The median pH was close to that of Kracie-61, and the pH

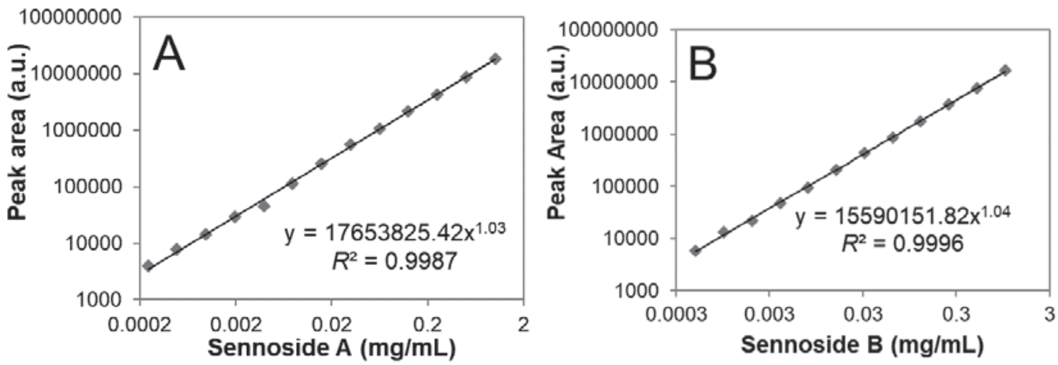

Figure 2. Standard curves for sennosides A and B.

Table 1. Amounts of Sennosides A and B in Kampo Medicines and Their Relative Strengths.

	Sennoside A (mg/pack)			Sennoside B (mg/pack)			Sennosides A and B (mg/pack)	Strength Scores
	Average		SEM	Average		SEM		
Junko-03	3.32	±	0.38	1.44	±	0.08	4.76	4.0
Kotaro-03	1.70	±	0.02	1.94	±	0.02	3.63	3.0
Kracie-03	1.78	±	0.03	1.99	±	0.01	3.77	3.1
Ohsugi-03	1.24	±	0.02	1.21	±	0.02	2.45	2.0
Taikodo-03	1.08	±	0.00	1.00	±	0.02	2.08	1.7
Teikoku-03	1.10	±	0.01	0.96	±	0.05	2.07	1.7
Tsumura-03	0.47	±	0.08	0.86	±	0.08	1.33	1.1
Taikodo-51	1.07	±	0.00	1.29	±	0.02	2.36	2.0
Tsumura-51	3.84	±	0.08	2.27	±	0.21	6.11	5.1
Junko-61	4.48	±	0.04	3.10	±	0.10	7.58	6.3
Kotaro-61	5.09	±	0.15	3.75	±	0.11	8.83	7.4
Kracie-61	6.64	±	0.04	5.04	±	0.02	11.68	9.7
Ohsugi-61	3.98	±	0.06	2.23	±	0.28	6.21	5.2
Teikoku-61	1.53	±	0.05	1.30	±	0.05	2.83	2.4
Tsumura-61	3.82	±	0.02	3.30	±	0.08	7.12	5.9
Tsumura-62	0.77	±	0.02	3.73	±	0.37	4.50	3.8
Kotaro-126	6.42	±	0.07	4.10	±	0.06	10.52	8.8
Ohsugi-126	4.24	±	0.04	3.13	±	0.05	7.38	6.1
Tsumura-126	2.56	±	0.14	2.16	±	0.05	4.72	3.9
Tsumura-134	1.23	±	0.07	1.17	±	0.07	2.39	2.0

The amount of sennosides A and B is the sum of the amounts of the two sennosides, but some of these are not equal to the sum of the two sennosides because of values less than significant figures.

of each sample solution was thus statistically compared with that of Kracie-61. The pH values of Junko-61 and Ohsugi-61 were significantly higher than that of Kracie-61, and those of Kotaro-61, Teikoku-61, and Tsumura-61 were significantly lower than that of Kracie-61 (Table 2).

The pH of the Masiningan aqueous solutions was 5.42-6.07. These pH values were statistically compared with those of Kotaro-126 and Ohsugi-126, with Tsumura-126 as a control, and the pH of Kotaro-126 was significantly higher than that of Tsumura-126; there was no significant difference between Ohsugi-126 and Tsumura-126 (Table 2).

The pH of Junchoto aqueous solutions was 5.21-5.26. These pH values were statistically compared with each other using Student's t-test, and there was a significant difference between them (p = 0.033). However, the significance was lost when the number of samples was increased (Table 2).

Discussion

Analyses of sennosides A and B in rhubarb-containing Kampo medicines indicated different amounts for sennosides A and B even when the formulas were the same (Table 1). For instance, the highest score for Tokakujokito was 9.7 (Kracie-61), and the lowest was 2.4 (Teikoku-61), with a nearly four-fold difference.

Some formulas (e.g., Otsujito, Junchoto, and Mashinin-gan) contained more than double the amount of sennosides A and B, even though they had the same formulas. The compositions of herbal medicines in each formula (i.e. the amounts of medicinal rhubarb in the formula) varied by brand (Table 3). This could be one reason for the difference in the amounts of sennosides A and B in the same formulas.

However, this alone is not enough to explain the difference in the amounts of sennosides A and B; therefore, it is necessary to consider other factors.

Another possibility is extraction conditions. The extraction methods for each brand should generally be similar, but differences such as hardness, pH, and extraction temperature could have some effect. Therefore, the formulas for Tsumura were compared to consider differences in extraction conditions among the 12 medicinal rhubarb-containing formulas.

The estimated strength scores for each formula were based on that for Daiokanzoto (Da Huang Gan Cao-Tang) obtained in our previous study and were analysed using HPLC[15]; formulas analysed in our previous study are illustrated in italics in Table 4.

When Tsumura's formulas analysed in this study were compared, Tsumura-61 had the highest score, suggesting that Tsumura-61 is stronger than Tsumura-84 Daiokanzoto[15]. A weak positive correlation was observed between Tsumura's formulas, except for Tsumura-84, and the medicinal rhubarb in those formulas ($R = 0.6000$).

However, different scores were observed for formulas

Table 2. pH of Aqueous Sample Solutions.

Sample	pH	Sample	pH
Junko-03	5.36 ± 0.02	Kotaro-61	5.58 ± 0.02
Kotaro-03 *	5.49 ± 0.01	*Kracie-61*	*5.56 ± 0.02*
Kracie-03	5.40 ± 0.02	Ohsugi-61 +	5.49 ± 0.01
Ohsugi-03	*5.37 ± 0.02*	Teikoku-61 ++	5.69 ± 0.01
Taikodo-03	5.35 ± 0.01	Tsumura-61 ++	5.66 ± 0.02
Teikoku-03 *	4.96 ± 0.01	Tsumura-62	5.27 ± 0.02
Tsumura-03 *	5.51 ± 0.01	Kotaro-126 #	6.07 ± 0.05
Taikodo-51	5.21 ± 0.01	Ohsugi-126	5.42 ± 0.02
Tsumura-51	5.26 ± 0.02	*Tsumura-126*	*5.42 ± 0.03*
Junko-61 ++	5.31 ± 0.02	Tsumura-134	5.12 ± 0.04

Data are expressed as means ± SEMs (n = 3). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's test. * $p < 0.01$ vs. Ohsugi-03, + $p < 0.05$, ++ $p < 0.01$ vs. Kracie-61, and # $p < 0.01$ vs. Tsumura-126. *Italics*: sample used as a control in each formula (sample number > 2).

Table 3. Medicinal Rhubarb and Other Laxative Components in Each Formula.

Agents	Rhubarb per package (g)	Other laxative components (g)	
Junko-03	0.33	none	
Kotaro-03			
Kracie-03			
Ohsugi-03			
Taikodo-03			
Teikoku-03			
Tsumura-03	0.17		
Taikodo-51	1.00	Hemp fruit 0.67	
Tsumura-51	0.67		
Junko-61	1.00	Anhydrous sodium sulphate	0.30
Kotaro-61			
Kracie-61			0.33
Ohsugi-61			0.30
Teikoku-61			0.67
Tsumura-61			0.30
Tsumura-62	0.50	Anhydrous sodium sulphate 0.23	
Kotaro-126	1.33	Hemp fruit 1.67	
Ohsugi-126			
Tsumura-126			
Tumura-134	0.67	none	

Table 4. Comparison of the Estimated and Analysed Strength Scores of Tsumura's Medicinal Rhubarb-containing Formulas Based on Their Daiokanzoto.

Estimated score	Analysed score	Brands	Formulas	Medicinal rhubarb (g)
0.54	1.11	Tsumura-03	Otsujito	0.17
1.07	3.97	Junko-03		0.33
	3.03	Kotaro-03		
	3.14	Kracie-03		
	2.04	Ohsugi-03		
	1.73	Taiko-03		
	1.72	Teikoku-03		
1.36	3.75	Tsumura-62	Bofutsushosan	0.50
2.17	5.10	Tsumura-51	Junchoto	0.67
	1.99	Tsumura-134	Keishikashakuyakudaioto	
3.25	5.93	Tsumura-61	Tokakujokito	1.00
	6.31	Junko-61		
	7.36	Kotaro-61		
	9.73	Kracie-61		
	5.18	Ohsugi-61		
	2.36	Teikoku-61		
	1.97	Taikodo-51	Junchoto	
4.33	*4.33*	*Tsumura-84*	*Daiokanzoto*	1.33
	8.8	Kotaro-126	Masiningan	
	6.1	Ohsugi-126		
	3.93	Tsumura-126		

with the same amount of medicinal rhubarb (Tsumura-51 and Tsumura-134).

The present extraction and formulation technologies are not extremely different; therefore, the temperature when those formulas are extracted should have fewer effects on the sennosides they contain.

Sennosides have low solubility in water at neutral pH, so it was considered that saponins or lipids would be good for sennoside extraction. However, no positive correlation was observed between the score and the amount of saponin-containing herbal medicines or between the score and the amount of lipid-containing herbal medicines.

The pH of solutions might have affected their extraction efficiency. In particular, sennosides were easily extracted under alkaline conditions, such as when sennosides were extracted using a sodium hydrogen carbonate solution in this study. However, the pH of all sample solutions was in the range of 5-6. The pH of some sample solutions might have been affected by magnesium aluminum silicate. Magnesium aluminum silicate was a basic compound found in all of Kotaro's agents and had a neutralising effect on the acidic solution[16]. This could lead to a slightly high pH in Kotaro's agents. A strong correlation was observed between the laxative strength scores and the pH of samples for Mashiningan (R = 0.8959) but not for Otsujito (R = 0.1727) and Tokakujokito (R = −0.2862). A significant correlation was also not observed among Tsumura's agents (R = 0.3105). Therefore, the pH of those samples could weakly affect the amounts of sennosides in Kampo medicines.

The above results suggested that the differences in strength scores might be caused by the quality of medicinal rhubarb, such as its botanical origins, but not by the other herbal medicines contained in those formulas. In JP, medicinal rhubarb is obtained from *R. palmatum, R tanguticum, R officinale,* and *R. coreanum* and their hybrids[2]. Some of these are called Kimmondaio (Jin Wen Da Huang) and Gao (Ya Huang), and they contained low amounts of sennosides compared with Shinshudaio, which is medicinal rhubarb hybridised and cultivated in Japan that has a high amount of sennosides[1]. The amounts of sennosides in Kinnmondaio and Gao are about one-fifth to half of those in Shinshudaio.

Differences in the amount of sennosides in medicinal rhubarb are likely due to Rheum species and their hybrids or the cultivation conditions. JP states that 1 g of dried rhubarb contains at least 2.5 mg of sennoside A[2].

However, both sennoside A and sennoside B have laxative effects, so quantifying only sennoside A according to JP would not provide adequate information for assessing the potential for long-term clinical use of medicinal rhubarb and Kampo medicines containing sennosides A and B. In addition, there is no information available about the upper limits of sennoside A and the total amounts of sennosides A and B. This could be one of reasons that sennoside amounts differed substantially among several Kampo medicines.

Some patients complained that laxative Kampo medicines had weaker effects or some unpleasant adverse reactions. Melanosis coli developed weaker effects when used for a long term. Even if not used for a long time, the Kampo medicines could have been produced from rhubarbs with lower quality, leading to abdominal pain. Kampo medicines would made using low-quality rhubarb containing rhaponticin, which causes abdominal pain.

The results obtained in this study should be similar for agents from the same batch; however, it is unclear whether similar results will be obtained for different batches of the same formula. The formulas may contain different amounts of sennosides detected in other batches. Therefore, in future research, we will analyse the amounts of sennosides in different batches of the same formulas.

Some kinds of rhubarb, such as Kinnmondaio, Gao, and Shinshudaio used in Kampo medicines, are still hard to identify using analytical techniques. Because they are boiled and extracted to prepare them as Kampo agents, their DNAs are cut off, and it thus becomes difficult to read the gene sequences. However, there is a possible way of identifying rhubarb's quality in a rhaponticin analysis.

The stilbenoid glycoside rhaponticin has been detected in some low-quality medicinal rhubarb[1]. Therefore, this might be useful as a marker compound for low-quality rhubarb[17]. However, it would be difficult to analyse sennosides and rhaponticin using a UV/Vis detector at the same time because of differences in the wavelength of maximum absorption between sennosides (λ_{max} = 260-270 nm)[18] and rhaponticin (λ_{max} = 343 nm)[19]. It might be possible to use an HPLC system equipped with a photodiode array detector, but such a system is quite expensive and, therefore, not always available for researchers when analysing these compounds.

An anti-rhaponticin monoclonal antibody might also be useful for qualitative and quantitative analyses of rhaponticin in Kampo medicines using enzyme-linked immunosorbent assay, even if the amount of rhaponticin is not sufficient for HPLC analysis. The detection method would make it clear whether low-quality rhubarb was used as an ingredient in Kampo medicines.

In this study, the laxative strength scores of 20 Kampo medicines were calculated. Those scores could potentially be used as a reference when physicians choose stimulant laxatives. However, some problems remain because it is not clear whether the strength of the laxatives is truly evaluated using the amounts of sennosides A and B alone.

Kampo medicines contain some other herbal medicines with laxative effects, such as the osmotic laxative sodium sulphate (Mang Xiao) and the miscellaneous laxative hemp fruit (Ma Zi Ren). Hemp fruit contains neutral lipids that are hydrolysed into glycerol and aliphatic acids[20]. Glycerol is an osmotic and lubricant laxative, and aliphatic acid is a lubricant laxative. The colonic transit time of patients administered Kampo medicines containing rhubarb and other laxatives might be shorter compared with that of patients administered Kampo medicines containing only rhubarb.

We analysed the colonic transit time of casanthranol and dioctyl sodium sulphosuccinate in our previous study[21]. In that study, we also used the Bristol stool form scale to evaluate stool form. The colonic transit time has also been evaluated using the Bristol stool form scale in other countries[22]. However, few clinical studies of rhubarb-containing Kampo medicines have been conducted to obtain data on colonic transit time and Bristol stool form scale, with the exception of Daikenchuto (Da Jian Zhong-Tang)[23,24]. Clinical tests involving administration of Kampo medicine to analyse colonic transit time and the Bristol stool form scale might enable application of the laxative strength score as a measure of total laxative effect. These refined scores would indicate the total strength of laxatives regardless of their mechanisms, which would help physicians and patients choose the most appropriate agents while minimising the use of stimulant laxatives, similarly to the analgesic opioid ladder and rotation approach used in terminal care[25].

Analyses of sennoside amounts in the following 10 Kampo medicines will be a future task: Daisaikoto (Da Chai Hu-Tang, 08), Daiobotampito (Da Huang Mu Dan Pi-Tang, 33), Jizusoippo (Zhi Tou Chuang-Yi Fang, 59), Choijokito (Tiao Wei Cheng Qi-Tang, 74), Jidabokuippo (Zhi Da Pu-Yi Fang, 89), Tsudosan (Tong Dao-San, 105), San'oshashinto (San Huang Xie Xin-Tang, 113), Daijokito (Da Cheng Qi-Tang, 133), Inchinkoto (Yin Chen Hao-Tang, 135), and Kumibinroto (Jiu Wei Bing Lang-Tang, 311)[26]. Furthermore, the formulas examined in this study will be analysed for different brands to create a better ranking of stimulant laxatives.

In conclusion, the results of this study suggest that the laxative strength score and the present ranking could assist physicians in more easily choosing appropriate medicinal rhubarb-containing Kampo medicines for use as laxatives, thereby helping patients relieve constipation without the adverse effects caused by anthraquinone analogues.

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Conflicts of Interest
There are no conflicts of interest.

Author Contributions
Ideas and Design of the Study: Keisuke Okazaki, Osamu Morinaga
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Data capturing using HPLC: Kakuyou Ogawa and Ai Tashima
Analyses and interpretation on the data: Ogawa Kakuyou, Keisuke Okazaki, and Osamu Morinaga
Approval of the final manuscript: Osamu Morinaga

Approval by Institutional Review Board (IRB)
none (This study does not include an approval code of IRB, because there are no data obtained from patients or any human beings and animals in the study.)

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