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NOTE

Effect of Schisandrae Fructus on glycyrrhizin content in Kampo extracts containing Glycyrrhizae Radix used clinically in Japan

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Abstract

Glycyrrhizae Radix is an important crude drug in Japan and is the most frequently prescribed drug in Kampo medicines for the treatment of a wide range of diseases. Glycyrrhizin (GL), the major active ingredient of Glycyrrhizae Radix, has various pharmacological actions but causes adverse effects such as pseudoaldosteronism. In a previous study, the GL content of shoseiryuto was found to be unexpectedly low, and Schisandrae Fructus in shoseiryuto reduced the pH value of the decoction and drastically decreased the extraction efficiency of GL from Glycyrrhizae Radix. In the present study, we investigated the extraction efficiency of GL from Glycyrrhizae Radix in decoctions comprising Glycyrrhizae Radix and five different fruit-derived crude drugs. Among the five fruit-derived crude drugs tested, Schisandrae Fructus markedly decreased both the pH value of the decoction and the extraction efficiency of GL. A comparison of the pH value of the decoction and the GL content of 12 Kampo prescriptions (containing at least Glycyrrhizae Radix and Schisandrae Fructus) showed that the GL content per daily dose was proportional to the compounding amount of Glycyrrhizae Radix, and that the extraction efficiency of GL from Glycyrrhizae Radix was strongly correlated with the pH value of the decoction. In addition, the pH value of the decoction was similar to the pH value documented in interview forms provided by pharmaceutical companies. These results suggested that the GL content in Glycyrrhizae Radix-containing Kampo products can be estimated from both the compounding amounts of Glycyrrhizae Radix and the pH value documented in their interview forms. Knowledge of GL content will help avoid adverse reactions due to Glycyrrhizae Radix.

Keywords Glycyrrhizae Radix · Glycyrrhizin · Kampo extracts · Schisandrae Fructus · pH · Pseudoaldosteronism

Introduction

Glycyrrhizae Radix is the most frequently used crude drug in Japan and is defined in the Japanese Pharmacopeia (JP) as the root and stolon of *Glycyrrhiza uralensis* Fischer or *Glycyrrhiza glabra* Linne [1]. Glycyrrhizae Radix is prescribed as an active component in multi-drug formulations of Kampo medicine to treat a variety of diseases.

Glycyrrhizin (GL), a major ingredient of Glycyrrhizae Radix, has various pharmacological actions [2–7] and is thought to be responsible for adverse effects such as pseudoaldosteronism [8–10]. As it is well-known that the high-dose and long-term administration of Glycyrrhizae Radix-containing Kampo medicines cause adverse effects, it is important to determine the GL content in Kampo medicines.

In our previous study, we evaluated 25 types of Kampo extracts containing Glycyrrhizae Radix and determined their GL content [11]. We found that the GL content per daily

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dose of each Kampo extract is generally proportional to the compounding amount of Glycyrrhizae Radix. In addition, the extraction efficiency of GL in the decoction is not constant and is dependent on the decoction pH. One of the combined crude drugs that affects the pH value and GL content of the decoction is Schisandrae Fructus, which is derived from the fruit and contains organic acids [11, 12]. However, the effects of combinations with other crude drugs prepared from the fruit on the pH value and extraction efficiency of GL of the decoction are unknown.

In the present study, we evaluated five types of typical crude drugs prepared from fruit compounds in Kampo formulas and combined with Glycyrrhizae Radix, and then measured the pH value and GL content of the decoction. We also investigated the pH value and GL content of all prescriptions involving the compounding of Glycyrrhizae Radix and Schisandrae Fructus approved in Japan and the relationship between the compounding amount of Glycyrrhizae Radix or pH value of the decoction and GL content. Moreover, we developed a method to estimate the GL content in ethical Kampo extract formulation using information included in interview forms (IFs).

Materials and methods

Materials

The crude drugs used to prepare the Kampo prescription were purchased from Tsumura & Co. (Ibaraki, Japan), Tochimoto Tenkaido Co., Ltd. (Osaka, Japan) and Daiko Shoyaku Ltd. (Aichi, Japan). All crude drugs used in this study were Japanese Pharmacopoeia 16th edition (JP XVI) grade. The same lot listed in Supplementary Table S1 was used in all experiments. High-performance liquid chromatography (HPLC)-grade acetonitrile, other solvents, and chemicals were purchased from Wako (Osaka, Japan). A syringe-driven filter unit (Millex-HP, 0.45 μm pore size) was purchased from Merck Millipore, Ltd. (Darmstadt, Germany). The standard GL (purity > 94%, HPLC) was kindly provided by Dr. Yukio Ogihara, Emeritus Professor at Nagoya City University.

Preparation of extracts of Glycyrrhizae Radix and other crude drugs prepared from the fruit and measurement of the pH values of decoctions

Glycyrrhizae Radix and other crude drugs prepared from the fruit were mixed in a 1:1 mass ratio (3 g each) and decocted with 600 mL ion-exchanged and distilled water using an electric heater (HMJ-1000N, HARIO Co., Ltd., Tokyo, Japan) for 60 min. The decoction was filtered and then cooled to around 25 °C. The pH value was measured with a pH meter (SevenEasy pH, Mettler Toledo, Columbus, OH, USA) using an electrode (InLab Expert Pro, Mettler Toledo). The filtrate was lyophilized to a powder and the extract was stored at − 20 °C until use.

Preparation of Kampo extracts containing Glycyrrhizae Radix with Schisandrae Fructus and measurement of the pH values of decoctions

The daily dosage of crude drugs compounded according to each Kampo formula listed in Table 1 was decocted with 600 mL ion-exchanged and distilled water using an electric heater for 60 min. The decoction was filtered and then cooled to room temperature. The pH value was measured with a pH meter using an electrode. The filtrate was lyophilized to a powder and stored at − 20 °C until use.

HPLC analysis of the GL content in extracts of Glycyrrhizae Radix and other crude drugs prepared from the fruit and 12 types of Kampo extracts containing Glycyrrhizae Radix with Schisandrae Fructus

All procedures were based on JP XVI. Briefly, 50 mg of each powdered extract was weighed and dissolved in water to a volume of 50 mL. The mixture was passed through a Millex-HP filter and subjected to HPLC analysis. The Shimadzu LC-10Avp HPLC series with UV–vis detector and C-R8A (Kyoto, Japan) was used for data acquisition and integration. Separations were conducted on an Inertsil-ODS3 (5 μm, 4.6 mm ID × 150 mm, GL Science, Tokyo, Japan) with 2% acetic acid–acetonitrile (60:40) as the eluent. The detection wavelength was 254 nm, the flow rate was 1.0 mL/min, and the column temperature was 40 °C. The GL content was determined using the absolute calibration curve method.

Statistical analysis

The results are given as the mean ± SEM. With respect to the effects of crude drugs prepared from the fruit on the pH values of the decoction and extraction efficiency of GL, statistical analysis was conducted by ANOVA using Bonferroni's multiple correction. Correlation analyses were performed using Pearson's correlation.

Table 1 The 12 types of Kampo prescriptions evaluated

Seinetsuhokito (Seinetsu-hoki-To)	
Ginseng Radix	3.0
Atractylodis Rhizoma	3.0
Poria	3.0
Angelicae Radix	3.0
Paeoniae Radix	3.0
Cimicifugae Rhizoma	1.0
Schisandarea Fructus	1.0
Scrophulariae Radix	1.0
Ophiopogonis Radix	3.0
Glycyrrhizae Radix	1.0
Ninjinyoeito (Ninjin-yoei-To)	
Ginseng Radix	3.0
Angelicae Radix	4.0
Paeoniae Radix	2.0
Rehmanniae Radix	4.0
Atractylodis Rhizoma	4.0
Poria	4.0
Cinnamomi Cortex	2.5
Astragali Radix	1.5
Citri Unshu Pericarpium	2.0
Polygalae Radix	2.0
Schisandrae Fructus	1.0
Glycyrrhizae Radix	1.0
Seihaito (Seihai-To)	
Scutellariae Radix	2.0
Platycodi Radix	2.0
Mori Cortex	2.0
Armeniacae Semen	2.0
Gardeniae Fructus	2.0
Asparagi Radix	2.0
Fritillariae Bulbus	2.0
Citri Unshu Pericarpium	2.0
Ziziphi Fructus	2.0
Bambusae Caulis	2.0
Poria	3.0
Angelicae Radix	3.0
Ophiopogonis Radix	3.0
Schisandrae Fructus	1.0
Zingiberis Rhizoma	1.0
Glycyrrhizae Radix	1.0
Kyososan (Kyoso-San)	
Perillae Herba	3.0
Schisandrae Fructus	2.0
Armeniacae Semen	2.0
Arecae Pericarpium	2.0
Mune Fructus Pareparatus	2.0
Asteris Radix	1.0
Platycodi Radix	1.0
Mori Cortex	1.0
Glycyrrhizae Radix	1.0
Fuhishomyakuyakusan (Fuhi-shomyaku-San)	
Ginseng Radix	1.5
Angelicae Radix	4.0
Paeoniae Radix	3.0
Asteris Radix	2.0
Astragali Radix	2.0
Ophiopogonis Radix	6.0
Schisandrae Fructus	1.5
Glycyrrhizae Radix	1.5
Seishoekkito (Seisho-ekki-To)	
Ginseng Radix	3.0
Atractylodis Rhizoma	3.0
Ophiopogonis Radix	3.0
Angelicae Radix	3.0
Astragali Radix	3.0
Citri Unshu Pericarpium	2.0
Schisandrae Fructus	2.0
Phellodendri Cortex	2.0
Glycyrrhizae Radix	2.0
Ryokankyomishingeninto (Ryo-kan-kyo-mi-shin-ge-nin-To)	
Poria	4.0
Glycyrrhizae Radix	2.0
Pinelliae Tuber	4.0
Zingiberis Rhizoma Processum	2.0
Armeniacae Semen	4.0
Schisandrae Fructus	3.0
Asiasari Radix	2.0
Ryokeimikanto (Ryo-kei-mi-kan-To)	
Poria	6.0
Cinnamomi Cortex	4.0
Glycyrrhizae Radix	2.0
Schisandrae Fructus	3.0
Shoseiryutogomakyoukansekito (Sho-seiryu-To-go-Ma-kyo-kan-seki-To)	
Ephedra Herba	4.0
Paeoniae Radix	3.0
Zingiberis Processum Rhizoma	3.0
Glycyrrhizae Radix	3.0
Cinnamomi Cortex	3.0
Asiasari Radix	3.0
Schisandrae Fructus	3.0
Pinelliae Tuber	6.0
Armeniacae Semen	4.0
Gypsum Fibrosum	10.0
Shoseiryutokasekko (Sho-seiryu-To-ka-sekko)	
Ephedra Herba	3.0
Paeoniae Radix	3.0
Zingiberis Processum Rhizoma	3.0
Glycyrrhizae Radix	3.0
Cinnamomi Cortex	3.0
Asiasari Radix	3.0
Schisandrae Fructus	3.0

Table 1 (continued)

Citri Unshu Pericarpium	1.0	Pinelliae Tuber	6.0
Ephedra Herb	1.0	Gypsum Fibrosum	5.0
Asini Corii Collas	1.0	Shoseiryuto (Sho-seiryu-To)	
Kamiuntanto (Kami-untan-To)		Ephedra Herba	3.0
Pinelliae Tuber	4.0	Paeoniae Radix	3.0
Poria	2.0	Zingiberis Processum Rhizoma	3.0
Citri Unshu Pericarpium	2.0	Glycyrrhizae Radix	3.0
Bambusae Caulis	2.0	Cinnamomi Cortex	3.0
Ziziphi Semen	3.0	Asiasari Radix	3.0
Schisandrae Fructus	3.0	Schisandrae Fructus	3.0
Polygalae Radix	3.0	Pinelliae Tuber	6.0
Ginseng Radix	3.0		
Rehmanniae Radix	3.0		
Ziziphi Fructus	2.0		
Aurantii Fructus Immaturus	2.0		
Zingiberis Rhizoma	1.0		
Glycyrrhizae Radix	1.0		

Results and discussion

Effects of crude drugs prepared from the fruit on decoction pH and glycyrrhizin extraction efficiency

We previously reported that the combination of Schisandrae Fructus reduced the pH of the decoction and drastically decreased the extraction efficacy of GL from Glycyrrhizae Radix [11]. Therefore, we investigated the effects of five crude drugs derived from different fruits (Ziziphi Fructus, Corni Fructus, Gardeniae Fructus, Euodiae Fructus and Schisandrae Fructus) on the pH of the decoction and extraction efficiency of GL.

As shown in Fig. 1, all crude drugs tested lowered the decoction pH compared with that of Glycyrrhizae Radix alone. Schisandrae Fructus showed the strongest influence on decoction pH. Corni Fructus also lowered the decoction pH, likely because of its high content of organic acids, such as malic acid, tartaric acid and gallic acid. However, all crude drugs studied, except for Schisandrae Fructus, had minimal effects on the extraction efficiency of GL from Glycyrrhizae Radix, although large variations were observed among three samples. Thus, the composition of Schisandrae Fructus in Glycyrrhizae Radix-containing Kampo formulas had a relatively lower GL content.

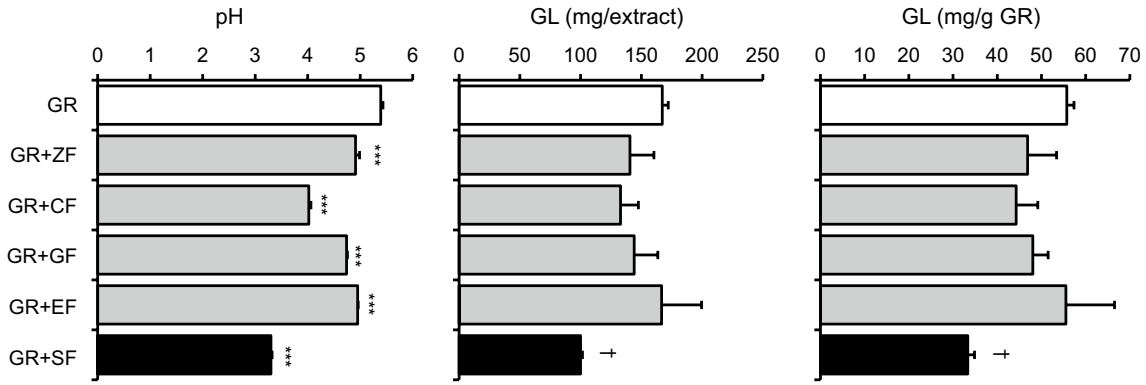

Fig. 1 The effects of crude drugs prepared from the fruit on the decoction pH and the glycyrrhizin (GL) extraction efficiency. *GR* Glycyrrhizae Radix alone, *GR* + *ZF* Glycyrrhizae Radix + Ziziphi Fructus, *GR* + *CF* Glycyrrhizae Radix + Corni Fructus, *GR* + *GF* Glycyrrhizae Radix + Gardeniae Fructus, *GR* + *EF* Glycyrrhizae Radix + Euodiae Fructus, *GR* + *SF* Glycyrrhizae Radix + Schisandrae Fructus. Each column represents the mean ± SEM of three samples. ***$p < 0.001$ vs the GR group by ANOVA with Bonferroni's multiple correction. $^{\dagger}p < 0.05$ vs the GR group by ANOVA with Bonferroni's correction for the selected two groups

Table 2 Yield, glycyrrhizin content and pH of the 12 Kampo extracts

Kampo prescription	Glycyrrhizin content			
	Yield (g)	Daily dose (mg)	Glycyrrhizae Radix (mg/g)	pH
Seinetsuhokito (Seinetsu-hoki-To)	8.33 ± 0.01	46.32 ± 1.07	46.32 ± 1.07	4.50 ± 0.01
Ninjinyoeito (Ninjin-yoei-To)	10.25 ± 0.01	36.56 ± 4.11	36.56 ± 4.11	4.46 ± 0.02
Seihaito (Seihai-To)	11.02 ± 0.02	41.53 ± 1.58	41.53 ± 1.58	4.24 ± 0.01
Kyososan (Kyoso-San)	5.31 ± 0.01	20.13 ± 0.55	20.13 ± 0.55	3.83 ± 0.01
Kamiuntanto (Kami-untan-To)	8.90 ± 0.05	30.22 ± 1.92	30.22 ± 1.92	3.78 ± 0.01
Fuhishomyakusan (Fuhi-shomyaku-To)	10.16 ± 0.04	70.88 ± 5.72	47.25 ± 3.82	4.38 ± 0.01
Seishoekkito (Seisho-ekki-To)	9.09 ± 0.06	62.04 ± 1.79	31.02 ± 0.89	4.23 ± 0.01
Ryokankyomishingeninto (Ryo-kan-kyo-mi-shin-ge-nin-To)	3.74 ± 0.04	91.82 ± 4.10	45.91 ± 2.05	3.61 ± 0.03
Ryokeimikanto (Ryo-kei-mi-kan-To)	2.08 ± 0.03	32.71 ± 1.36	16.36 ± 0.68	3.28 ± 0.01
Shoseiryutogomakyoukansekito (Sho-seiryu-To-go-Ma-kyo-kan-seki-To)	6.54 ± 0.05	55.13 ± 4.85	18.38 ± 1.62	3.78 ± 0.03
Shoseiryutokasekko (Sho-seiryu-To-ka-sekko)	6.04 ± 0.13	64.42 ± 3.72	21.47 ± 1.24	3.71 ± 0.01
Shoseiryuto (Sho-seiryu-To)	5.41 ± 0.30	79.81 ± 6.03	26.60 ± 2.01	3.87 ± 0.03

Glycyrrhizin content in 12 Kampo extracts involving the compounding of Glycyrrhizae Radix with Schisandrae Fructus

Among the ethical and over-the-counter Kampo formulas approved by the Ministry of Health, Labour and Welfare, 12 types of Kampo formula compounding Glycyrrhizae Radix with Schisandrae Fructus are listed in Table 1. We prepared these Kampo extracts and measured the decoction pH and GL content of the Kampo extracts (Table 2). There was no correlation between the compounding amount of Glycyrrhizae Radix and the extraction efficiency of GL from the crude drug, although a weak correlation was detected between the compounding amount and GL content of each daily dose

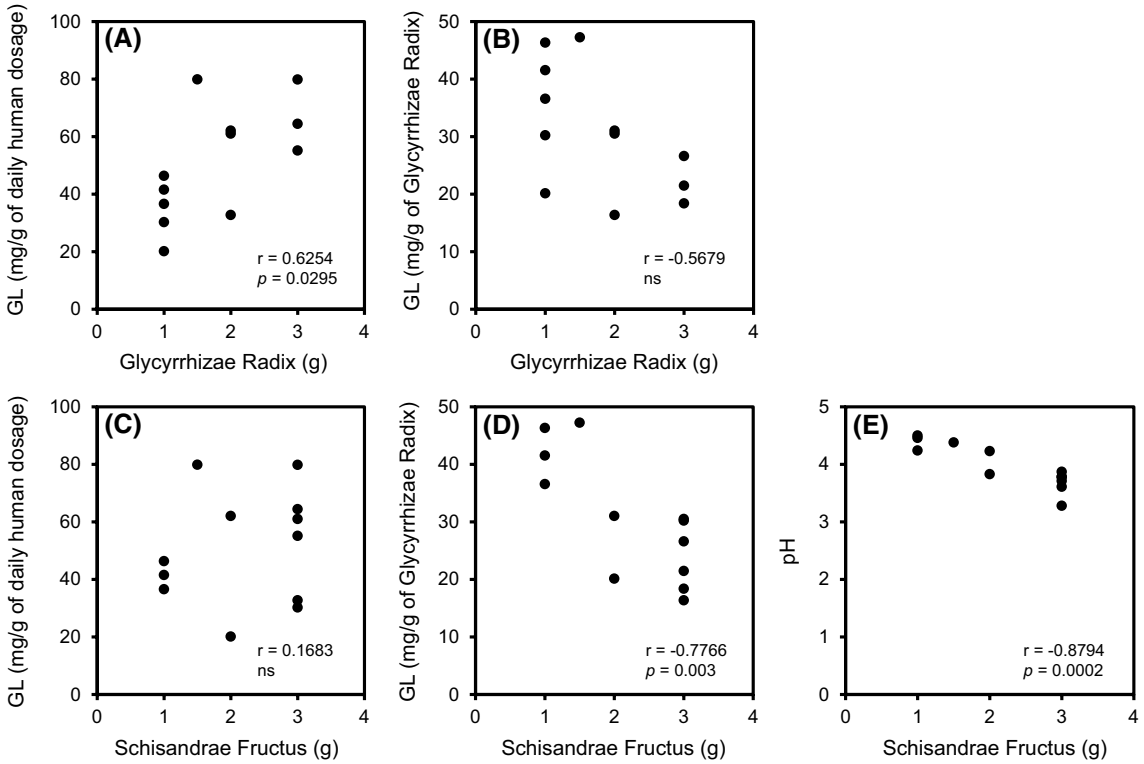

Fig. 2 Correlation analysis of the compounding amounts of Glycyrrhizae Radix or Schisandrae Fructus and the GL content or pH value of the decoctions for the 12 Kampo extracts

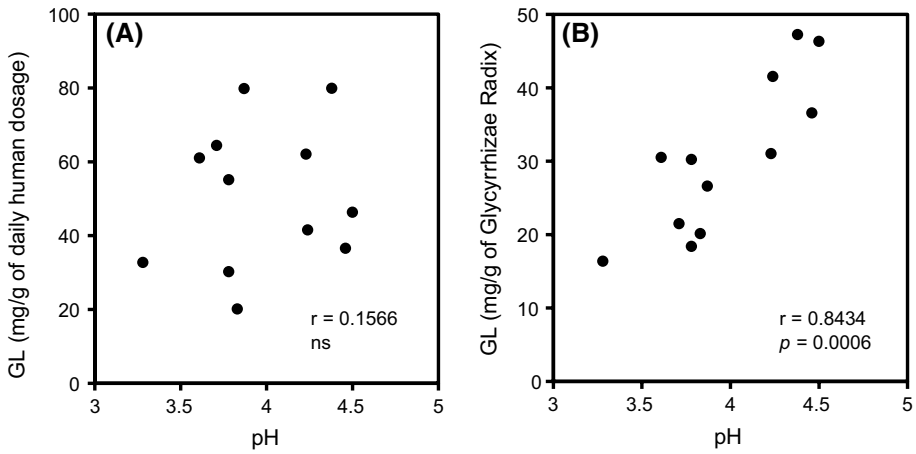

Fig. 3 Correlation analysis between the pH of decoctions and the GL content of the 12 Kampo extracts

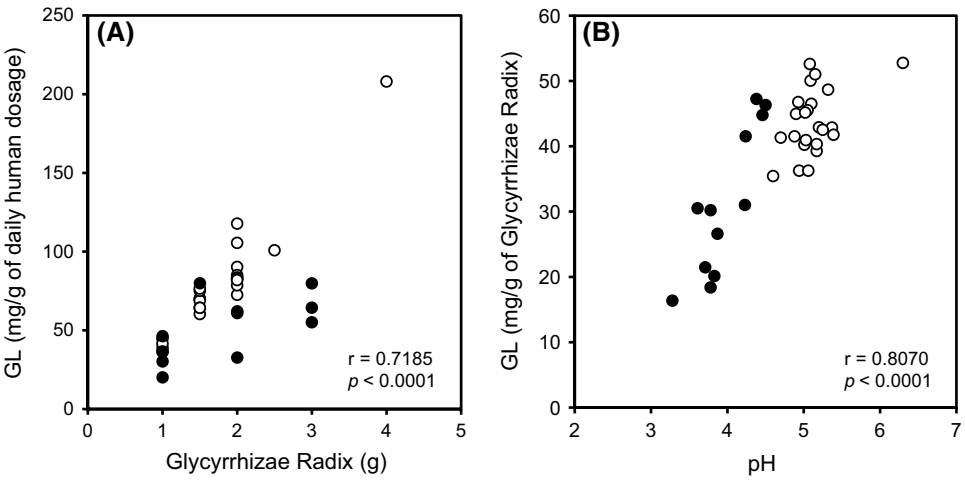

Fig. 4 Correlation analysis of 35 Kampo extracts containing Glycyrrhizae Radix between the compounding amounts of Glycyrrhizae Radix or decoction pH and the GL content (filled circle), Kampo extracts containing Glycyrrhizae Radix and Schisandrae Fructus tested in this study (unfilled circle), Kampo extracts containing Glycyrrhizae Radix tested in our previous study [11]

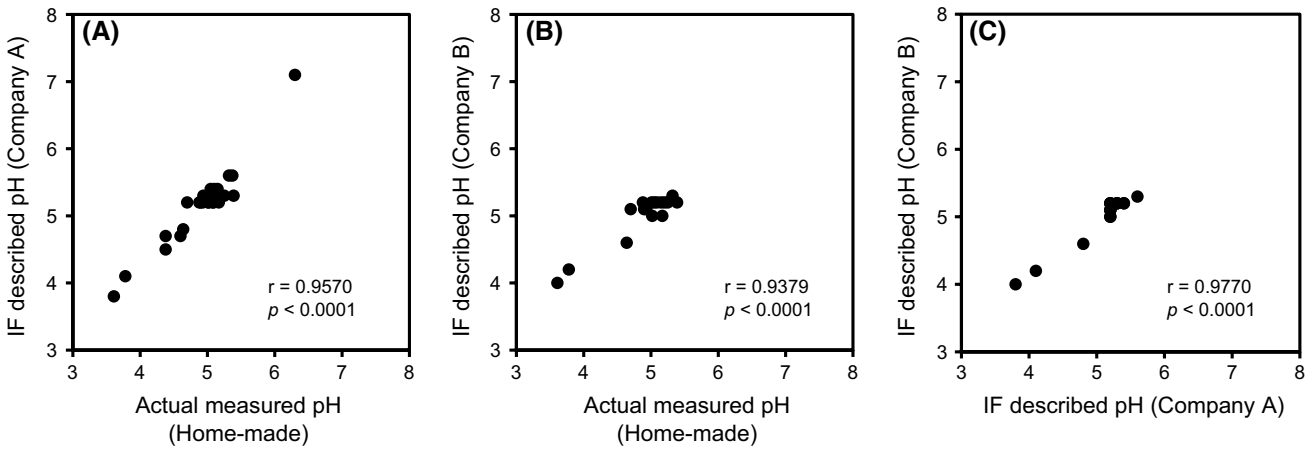

Fig. 5 Correlation analysis between decoction pH and pH described in interview forms provided by the pharmaceutical companies

(Fig. 2a, b). The combination with Schisandrae Fructus did not influence the GL content of each extract, but did affect the extraction efficiency of GL (Fig. 2c, d). The decoction pH gradually decreased when the compounding amount of Schisandrae Fructus was increased (Fig. 2e). These results suggest that the combination of Schisandrae Fructus lowered the decoction pH and decreased the GL extraction efficiency from Glycyrrhizae Radix. As shown in Fig. 3, there was a

good correlation between the pH value and the extraction efficiency of GL in 12 types of Kampo extracts.

As shown in Fig. 4, a good correlation was observed between the decoction pH and GL extraction efficiency ($r = 0.8070$, $p < 0.001$), as well as between the compounding amounts of Glycyrrhizae Radix and the GL content of the daily dose ($r = 0.7185$, $p < 0.0001$).

The pH value of the ethical Kampo extract formulations is available on the interview form (IF) as part of the detailed information provided for the package insert published by pharmaceutical companies. The pH values of the decoctions measured in our study were similar to those documented in the IFs provided by the representative companies (Fig. 5a, b). Moreover, the pH values shown by the companies were well-correlated with each other (Fig. 5c). It is well-known that the compounding ratio of crude drugs, and sometimes the crude drugs themselves, differed among companies, because there are many ancient literatures that each company refer to determine the prescriptions of Kampo formulas. However, more than one prescription can be used for each Kampo formula. The correlations shown in Fig. 5 are very impressive and suggest that each Kampo extract may have a certain pH value.

In this study, we tested the effects of typical fruit-derived crude drugs on GL extraction efficiency when those crude drugs were combined with Glycyrrhizae Radix and we confirmed the lowering actions of Schisandrae Fructus on the decoction pH and the GL extraction efficiency. In addition, we ascertained that the GL content per daily dose in Kampo medicine was generally proportional to the compounding amount of Glycyrrhizae Radix and that the GL extraction efficiency in the decoction was essentially dependent on the decoction pH in Glycyrrhiza Radix-containing Kampo extracts. These results suggested that the GL content in Glycyrrhizae Radix-containing Kampo products can be estimated from the amount of Glycyrrhizae Radix and the pH value documented in their IFs, which should help to avoid adverse reactions due to Glycyrrhizae Radix.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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