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Chemical and pharmaceutical evaluation of Daitou-Gancao in comparison with usual Glycyrrhizae Radices

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Abstract

The chemical and pharmaceutical characteristics of Daitou-Gancao (Taito-Kanzo in Japanese), which is a newly imported Chinese Glycyrrhizae Radix from about 1997 in order to make up for the deficit of Dongbei-Gancao (Tohoku-Kanzo in Japanese) was studied. Its botanical origin was identified as *Glycyrrhiza uralensis* FISCH., by on-the-spot investigations in the Gansu and Shanxi provinces of China. The HPLC fingerprint of Daitou-Gancao was similar to that of two concurrent medicinal Dongbei- and Xibei-Gancao (Seihoku-Kanzo), but different from that of Xinjiang-Gancao (Shinkyo-Kanzo), which does not conform to the Japanese Pharmacopoeia XIV standard. Daitou-Gancao and the medicinal Dongbei- and Xibei-Gancao were also clearly distinguishable from non-medicinal Xinjiang-Gancao by principal component analysis and hierarchical cluster analysis using 7 HPLC-peak-area data. Furthermore, Daitou-Gancao had an anti-allergic effect and pharmacokinetic profile of glycyrrhetic acid, an active metabolite of glycyrrhizin, similarly to Dongbei-Gancao. The present chemical and pharmaceutical study suggests that Daitou-Gancao could be used concurrently with Dongbei- and Xibei-Gancao, which conform to the JP XIV.

Key words *Glycyrrhiza uralensis*, glycyrrhizin, HPLC fingerprint, principal component analysis, allergic inflammation, pharmacokinetics.

Abbreviations AUC, area under the concentration versus time curve; DNFB, dinitrofluorobenzen; DNP, dinitrophenol; GA, glycyrrhetic acid; GL, glycyrrhizin; HCA, hierarchical cluster analysis; IPR, immediate phase response; LPR, late phase response; mAb, monoclonal antibody; C_{max}, maximum plasma concentration; PCA, principal component analysis; T_{max}, time to reach maximum plasma concentration; vLPR, very late phase response; Daitou-Gancao (Taito-Kanzo: 帯頭甘草); Dongbei-Gancao (Tohoku-Kanzo: 東北甘草); Xibei-Gancao (Seihoku-Kanzo: 西北甘草); Xinjiang-Gancao (Shinkyo-Kanzo: 新疆甘草).

Introduction

In the course of our pharmacognostical studies on Chinese Gancao (Kanzo in Japanese) resources, we previously revealed that Daitou-Gancao (Chinese commercial name; Taito-Kanzo in Japanese) has been imported into Japan since about 1997 in order to make up for a shortage of Dongbei-Gancao (Tohoku-Kanzo).[1] Daitou-Gancao collected in the confined regions of Gansu and Shanxi provinces of China is characterized by intermediate properties between Dongbei- and Xibei-Gancao (Seihoku-Kanzo).

Gancao, the underground parts of the *Glycyrrhiza* species, is a well-known traditional Chinese drug used for inflammatory, allergic and gastric disorders.[2] Three kinds of Gancao, Dongbei-Gancao (Dongbei in Chinese meaning northeast), Xibei-Gancao (Xibei meaning northwest) and Xinjiang-Gancao (Shinkyo-Kanzo; Xinjiang is a province name), are currently used in Japan. The former two Gancao are permitted to be used for medicinal purposes by the Japanese Pharmacopoeia XIV (JP XIV), whereas Xinjiang-Gancao does not meet the JP XIV standard and is used as a food additive and as a source for extracting glycyrrhizin (GL), one of the active ingredients of Gancao.[2]

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Gancao is most frequently prescribed in the traditional Chinese formulations described in Shan-Han-Lun,[3] a famous traditional Chinese formulary and plays an important role in the Japanese medical care system (called Kampo medicine). With the increasing demand for Gancao and decline of wild *Glycyrrhiza* resources from traditional regions in China, Daitou-Gancao will become an additional resource for medicinal Gancao. Therefore, a quality control study of newly imported Daitou-Gancao is necessary to assure safety and effective clinical use.

The aim of the present study was to clarify the botanical origin of Daitou-Gancao as well as to examine its chemical and pharmaceutical properties in comparison with those of medicinal Dongbei-Gancao. The chemical constituents of Daitou-Gancao were compared with those of 3 kinds of Gancao including Xinjiang-Gancao using HPLC fingerprints (chromatograms). The fingerprints were also examined quantitatively by the principal component analysis (PCA) and hierarchical cluster analysis (HCA) on the basis of HPLC-peak-area data. Furthermore, the pharmaceutical properties of Daitou-Gancao were compared with those of Dongbei-Gancao by examining anti-allergic effects on an IgE-mediated triphasic skin reaction in mice[4] and the pharmacokinetic fate of glycyrrhetic acid (GA),[5] an active metabolite of GL, from two Gancao extracts administered orally.

Materials and Methods

Gancao and G. uralensis: Daitou-Gancao (12 lots, produced in the Gansu province of China, collected in Shanxi province and market of Osaka in 2000), Dongbei-Gancao (7 lots, Nei-Meng-Gu in 1990, 1992, 1994, 1998 and 1999, Hebei in 1996 and Hong Kong market in 1991) and Xibei-Gancao (7 lots, Nei-Meng-Gu in 1991, Qinghai in 1992, Ningxia in 1993, 1994, 1998, and 2000, Shanxi in 1997) were used. Xinjiang-Gancao (Xinjiang and Hebei in 2000) possessing lichochalcone A were selected by examining the reported TLC analysis (TLC plate, Silica gel $60F_{254}$ MERCK No. 5715; Solvent, $CHCl_3$/MeOH/H_2O: 65:35:10; Detected at 365 nm)[6] before HPLC fingerprint analysis. These 4 kinds of Gancao are the trading names used in China. The same 4 wild *G. uralensis* roots as in the previous report[1] were used.[7]

Animals: Female specific pathogen-free BALB/c mice (6 weeks old: for the triphasic skin reaction study) and male Wistar rats (8 weeks old: for the pharmacokinetic study) were purchased from Japan SLC Inc., (Hamamatsu, Japan) and maintained in the Laboratory for Animal Experiments, Toyama Medical and Pharmaceutical University. All animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Chemicals: GL standard (control: No. 992) was purchased from the National Institute of Health Sciences, Japan, and GA and liquiritin were purchased from Wako Pure Chemicals Industries Ltd., Osaka. Liquiritin apioside and isoliquiritin apioside were kindly provided by Mr. Takayuki Nakada of Alps Pharmaceutical Ind., Co., LTD. Isoliquiritin was kindly provided by Dr. Kazuo Koike of Toho University, School of Pharmaceutical Sciences. Lichochalcone A was isolated from the peels of Xinjiang-Gancao by preparative TLC (TLC plate, Silica gel $60F_{254}$, MERCK No. 5717; Solvent, $CHCl_3$/MeOH/H_2O: 65:35:10; and re-chromatographed with $CHCl_3$/MeOH: 20:5 and n-hexane/AcOEt 20:5; Detected at 365 nm) and identified by comparison with the reported HPLC chromatogram.[8] Dinitrofluorobenzene (DNFB) and dinitrophenol (DNP) were purchased from Nacalai Tesque, Kyoto. Prednisolone-21 acetate was obtained from Sigma Chemical Co., St. Louis. All other chemicals and solvents were of analytical and/or HPLC grade.

HPLC fingerprint analysis: About 5 g of powder of each sample prepared by the procedure described in the legend of Fig. 3 was extracted using 90% aqueous MeOH (50 ml, under reflux for 1 hr for three times). The resulting MeOH extract was diluted to 250 ml with 50% aqueous EtOH and the soluble portion was filtered though a 0.45 μm filter and an aliquot (5 μl) of filtrate was analyzed by HPLC under the conditions[8] described in the legend of Fig. 3.

Multivariate analysis based on HPLC-peak-area data: The 7 peak-area data (**A** to **G**, A_n: n= 7, from **A** to **G** in Fig. 3) were calculated from autoscale preprocessing with the following equation: $(A_n - A_{mean})$ / (standard deviation: SD), where A_{mean} represents the mean value of each peak-area. Then PCA and HCA were examined using the multivariate analysis software (Pirouette™, Infometrix Co., Woodinville, USA).

Skin reaction in mouse ears: Daitou- and Dongbei-Gancao were extracted by boiling in water for 40 min and freeze-dried into a powder (yield of Daitou-Gancao: 1.04±0.02 g and Dongbei-Gancao: 0.87±0.03 g, from 3 g). In order to obtain the average for the yield and quality of batch, the operations were further repeated five times. The preparation of anti-DNP monoclonal antibody (mAb) IgE, estimation of IgE antibody titers and induction of skin reactions were carried out as previously reported.[9] Briefly, BALB/c mice were given a 1 ml aliquot of anti-DNP IgE mAb-containing fluid 24 h (an i.v. injection) before the DNFB challenge. A skin reaction was elicited by applying 10 μl of 0.1% DNFB/EtOH to each side of each ear of the sensitized mice. Ear swelling was evaluated using a dial thickness gauge (G-1A type, Peacock, Ozaki MFG., Co., Ltd., Osaka) immediately before and at appropriate times after the challenge.

Pharmacokinetic fate of GA: The same freeze-dried extracts of Daitou- and Dongbei-Gancao as used in the skin reaction were used. Plasma concentration-time profiles of GA were examined as previously reported.[5] Briefly, rats were fasted overnight prior to oral administration of Gancao extract (at a dose containing 45 mg/kg GL) and then blood samples (about 0.3 ml) were collected from the tail vein through heparin-coated micro capillaries at 1, 2, 4, 6, 9, 12 and 24 h after the administration. The samples were immediately centrifuged at 3000 rpm for 10 min to obtain the plasma, which was stored at -20℃ until analysis. The mixture of plasma sample (100 μl) and MeOH (200 μl) was centrifuged at 10,000 rpm for 10 min and the resulting supernatant was filtered through a 0.45 μm membrane filter. The filtrate (100 μl) was subjected to HPLC, the system and conditions for which were the same as in our previous report.[5]

Statistics: Results are shown as the mean ± S.D. For the skin reaction, the statistical significance of differences between groups was determined using the Mann-Whitney's U-test. Other data were evaluated statistically using Student's *t*-test performed for a comparison of the means. Probability (*p*) values less than 0.05 were considered significant.

Results and Discussion

Botanical origin of Daitou-Gancao (Figs. 1, 2)

Daitou-Gancao has a knot-like upper root (Daitou in Chinese meaning "with head"), and high GL-content as Dongbei-Gancao, and it has a firm root (high root specific gravity) as Xibei-Gancao.[1] In order to clarify the botanical origin of Daitou-Gancao, we investigated growing *Glycyrrhiza* plants in the Daitou-Gancao producing districts (Heshui, Wuqi and Zhidan in China: Fig. 1) in July and October 2001. The *Glycyrrhiza* plant specimens[10] collected at the producing districts were identified to be a *G. uralensis* FISCH. by referring to the described characteristics[11] of undulate leaf-lets and falcate-fruits with prickle hairs (Fig. 2). On the basis of the present on-the-spot investigation, it was confirmed that the botanical origin of Daitou-Gancao collected in the confined regions of Gansu and Shanxi provinces of China were in accordance with the JP XIV standard.

HPLC fingerprints of Daitou-Gancao and other Gancao (Figs. 3, 4)

As shown in HPLC fingerprints (Fig. 3), Daitou-Gancao have two peaks of liquiritin (peak **B**) and GL (**F**) as the major components detected at 254 nm (left panel) and two peaks of isoliquiritin apioside (**C**) and isoliquiritin (**D**) detected at 350 nm (right panel). The HPLC fingerprint was similar to that of two medicinal

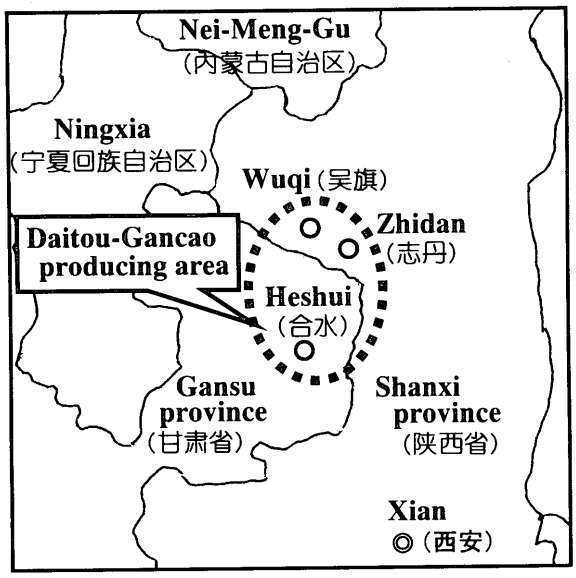

Fig. 1. On-the-spot investigation in Daitou-Gancao producing areas.
Daitou-Gancao is produced in the confined regions of northeast of Gansu province and northwest of Shanxi province of China. We made on-the-spot investigations at Heshui in Gansu, and Wuqi and Zhidan in Shanxi in July and October 2001 to collect original plant specimens of Daitou-Gancao.
The yearly output of Daitou-Gancao reaches about 500 tons in 2001 by our on-the-spot investigations at Gansu and Shanxi in 2001.

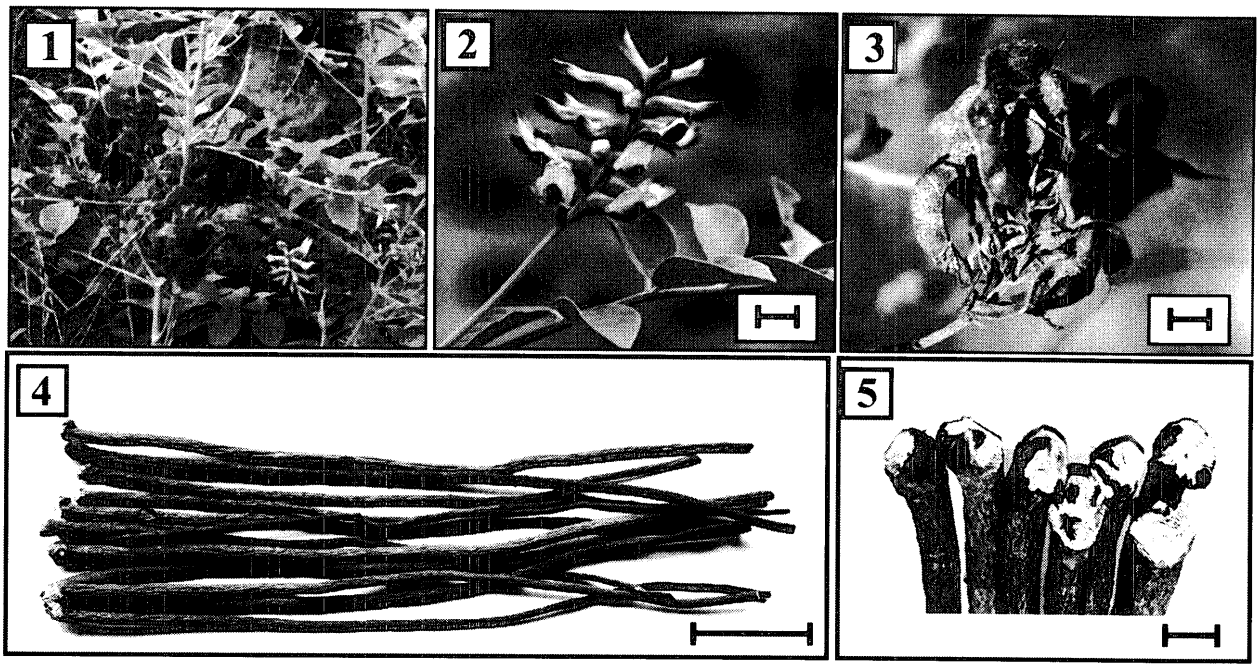

Fig. 2. Daitou-Gancao and its original plant (*G. uralensis*) collected in Gansu and Shanxi provinces.
1: flower and fruits; **2**: flower (Bar: 1 cm); **3**: fruits (Bar: 1 cm); **4**: whole shape of Daitou-Gancao. (Bar: 15 cm); and **5**: knot-like upper root (Bar: 3 cm).
Daitou-Gancao is similar to Dongbei-Gancao in shape of root and knot-like upper root.
1 and **2** were photographed at Heshui in Gansu province on 27-July-2001, and **3** was photographed at Zhidan of Shanxi province on 26-October-2001.

Dongbei- and Xibei-Gancao and wild *G. uralensis* roots (collected in the northeast area of Nei-Meng-Gu,[1] one of the major sources of Dongbei-Gancao).

On the other hand, the HPLC fingerprint of Xinjiang-Gancao[12] was characterized by peaks **E**, **Y**, **G** and **Z** as well as a major peak **F** (GL) detected at 254 nm in the left panel of Fig. 3 and major peaks **E** and **G** (lichochalcone A) detected at 350 nm in the right panel of Fig. 3. Peak **E** was estimated as lichochalcone B by referring to the reported HPLC profile.[8] These HPLC fingerprints coincided with those of previous reports.[8,13] The present HPLC fingerprint analysis indicated that the medicinal Gancao tested in this study was distinguishable from Xinjiang-Gancao, which does not meet the JP XIV standards. Thus, the fingerprint analysis is useful for quality control of crude drugs possessing many ingredients. Furthermore, it conforms well to the recent recommendation that chromatographic fingerprints be used as part of a chemical identification index for botanical products, prescribed by the Food and Drug Administration (FDA) of the U. S. Department of Health and Human Services.

The HPLC fingerprints detected at 254 nm in Gancao (the left panel of Fig. 3) were presented as bar code-like figures on the basis of the peak area and retention time of each peak. In Fig. 4, the thickness and position of each bar corresponds to the peak area and retention time of each peak. It is clear that the bar code-like figure of Daitou-Gancao was similar to that of medicinal Gancao and different from that of Xinjiang-Gancao. The **V** and **W** bars, which are unidentified peaks, observed in Daitou-, Dongbei- Xibei-Gancao and wild *G. uralensis* roots are absent (or present at trace levels) in non-medicinal Xinjiang-Gancao. In contrast, **E**, **Y**, **G** and **X** bars observed in Xinjiang-Gancao are absent (or present at trace levels) in Daitou-Gancao, two medicinal Gancao and wild *G. uralensis* roots. The present bar code-like figures may be used as a simple and easy way to compare medicinal with non-medicinal Gancao.

PCA and HCA using HPLC-peak area data on Gancao (Table I and Figs. 5, 6)

The qualitative fingerprint data were treated quantitatively by multivariate analysis on the basis of HPLC-peak-area data. As shown in the scatter diagram (Fig. 5) of PCA obtained using 7 peak-area data (peaks **A** to **G** in Fig. 3) as variables, Daitou-Gancao, two medicinal

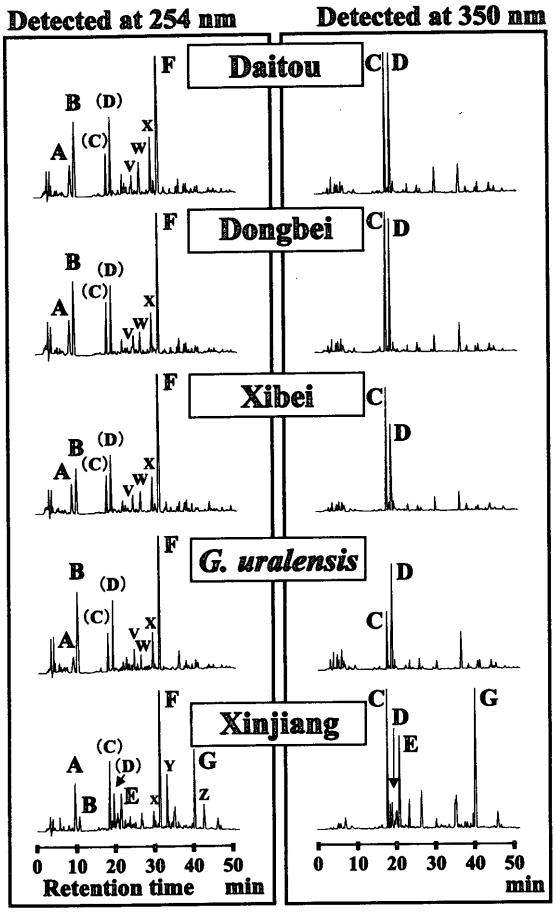

Fig. 3. HPLC fingerprints of 4 kinds of Gancao and *G. uralensis* roots.
For HPLC fingerprint analysis, three MeOH extracts prepared from about 400 whole roots of Daitou- (12 lots), Dongbei- (7 lots) and Xibei-Gancao (7 lots) and two MeOH extracts prepared from 7 and 4 whole roots of Xinjiang-Gancao and *G. uralensis* were used.
Left panel, profiles detected at 254 nm; Right panel, profiles detected at 350 nm.
HPLC conditions, Shimadzu LC-10AT with C-R7A and SPD-10A; Column, YMC-Pack ODS-AQ (AQ-312: 150 × 6 mm, YMC Co., Ltd.) set at 40℃; Mobile phase, 1% AcOH/CH_3CN (80:20)→5 min (80:20) →50 min (20:80); Flow-rate, 1.0 ml/min.
A, liquiritin apioside; **B**, liquiritin; (**C**) in left panel; isoliquiritin apioside and some peaks; **C**, isoliquiritin apioside; (**D**) in left panel; isoliquiritin and some peaks; **D**, isoliquiritin; **E**, (lichochalcone B estimated); **F**, glycyrrhizin; **G**, lichochalcone A; **V-Z**, unidentified peaks.

Gancao and wild *G. uralensis* roots can be distinguished mutually from Xinjiang-Gancao. The contribution of the first (Z1) and second (Z2) principal components obtained using the 7 peak-area data was 50.8 and 24.7%, respectively (Table I), which means that the first two principal components could account for 75.5% of the variance in the data. The present results obtained by PCA based on the HPLC-7-peak-area data will be a reliable method to discriminate medicinal Gancao from Xinjiang-Gancao. The PCA is considered a useful method to estimate the overall characteristics of natural drugs possessing

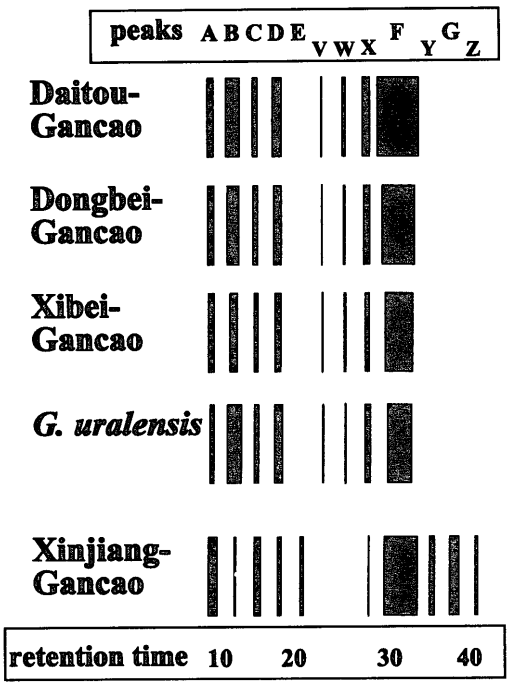

Fig. 4. Schematic diagrams (bar code) of HPLC fingerprints of 4 kinds of Gancao and *G. uralensis* roots.
HPLC fingerprints detected at 254 nm (the left panel of Fig. 3) were represented schematically to bar code on the basis of peak area ratio and retention time of each peak. The peaks possessing more than a 50,000-peak area score calculated by using C-R7A (Shimadzu) in Daitou-Gancao were selected and compared with other samples. Letters described in the upper side and figures in the lower side correspond to peak name and retention time in the HPLC fingerprints of Fig. 3, respectively.

Table I Contribution of the first (Z1), second (Z2) and third (Z3) principal component from HPLC 7 peak-area data

Variables	Z1	Z2	Z3
A: liquiritin apioside	0.373	0.328	−0.477
B: liquiritin	−0.452	0.313	0.091
C: isoliquiritin apioside	0.367	0.429	−0.396
D: isoliquiritin	−0.438	0.390	0.051
E: lichochalcone B (estimated)	0.381	0.061	0.579
F: glycyrrhizin	−0.045	0.668	0.304
G: lichochalcone A	0.427	0.093	0.422
Variance	163.5	79.7	55.1
Cumulative Contribution ratio (%)	50.8	75.5	92.6

A to **G**: peaks as shown in Fig. 3

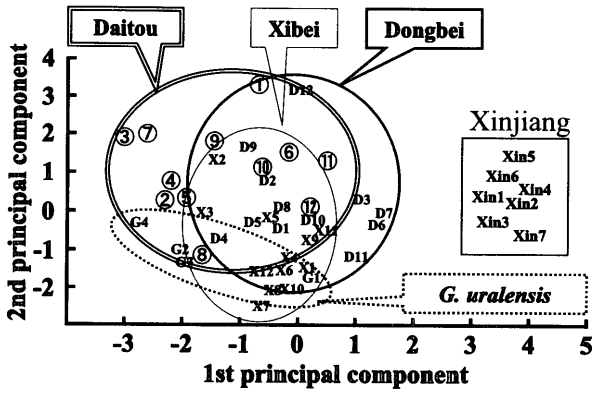

Fig. 5. Scatter diagram of principal component score (Z1, Z2) for 4 kinds of Gancao and *G. uralensis* roots on the basis of HPLC-7-peak-area data.
The figure represents a plot of the scores obtained by PCA for 1st and 2nd principal components of 4 Gancao and *G. uralensis* roots. The principal component data were described in Table I. PCA examination was carried out on the basis of HPLC-7-peak-area data (**A** to **G**) examined using MeOH extracts prepared from 12, 12, 12, 7 and 4 whole roots of Daitou-, Dongbei-, Xibei- and Xinjiang-Gancao and wild *G. uralensis*, respectively. ①-⑫, Daitou-Gancao (collected at Gansu in 2000, GL-content: 6.48 ± 1.18%; RSG: 0.56 ± 0.08 g/cm^3); D1-D12, Dongbei-Gancao (D1, Nei-Meng-Gu in 1990; D2-5, Hong Kong market in 1991; D6-8, Nei-Meng-Gu in 1992; D9, Nei-Meng-Gu in 1994; D10, Hebei in 1996; D11, Nei-Meng-Gu in 1998; D12, Nei-Meng-Gu in 1999; GL-content: 4.94 ± 1.31; RSG: 0.49 ± 0.04); X1-X12, Xibei-Gancao (X1, Nei-Meng-Gu in 1991; X2-3, Qinghai in 1992; X4, Ningxia in 1993; X5, Ningxia in 1994; X6, Shanxi in 1997; X7-8, Ningxia in 1998; X9-12, Ningxia in 2000; GL-content: 4.51 ± 1.27; RSG: 0.65 ± 0.13); Xin1-Xin7, Xinjiang-Gancao (Xinjiang and Hebei in 2000; GL-content: 5.81 ± 0.79; RSG: 0.67 ± 0.17); G1-G4, *G. uralensis* roots (eastern Nei-Meng-Gu in 2000; GL-content: 4.30 ± 0.99; RSG: 0.47 ± 0.01)

various morphological[14] and chemical ingredients.[15-16]

Furthermore, it was proved that the properties of Daitou-Gancao are similar to those of the two medicinal Gancao and *G. uralensis* roots in several trials of PCA and HCA using from 5 to 11 kinds of HPLC-peak-area-data. For example, as shown in the dendrogram obtained by HCA based on the HPLC-7-peak-area-data (Fig. 6), 4 Gancao and wild *G. uralensis* roots were divided clearly into cluster I which contains non-medicinal Xinjiang-Gancao and cluster II which contains Daitou-Gancao, two medicinal Gancao and wild *G. uralensis* roots. The cluster II had three sub-clusters such as II-1 composed mainly of Dongbei- and Xibei-Gancao, II-2 mainly comprising Daitou-Gancao and Dongbei-Gancao characterized by a larger peak **C** and **F** (GL), and II-3 formed mainly from Daitou-Gancao and wild *G. uralensis* roots. The present HCA showed that Daitou-Gancao has a similar HPLC fingerprint to those of the two medicinal Gancao and wild *G. uralensis* roots.

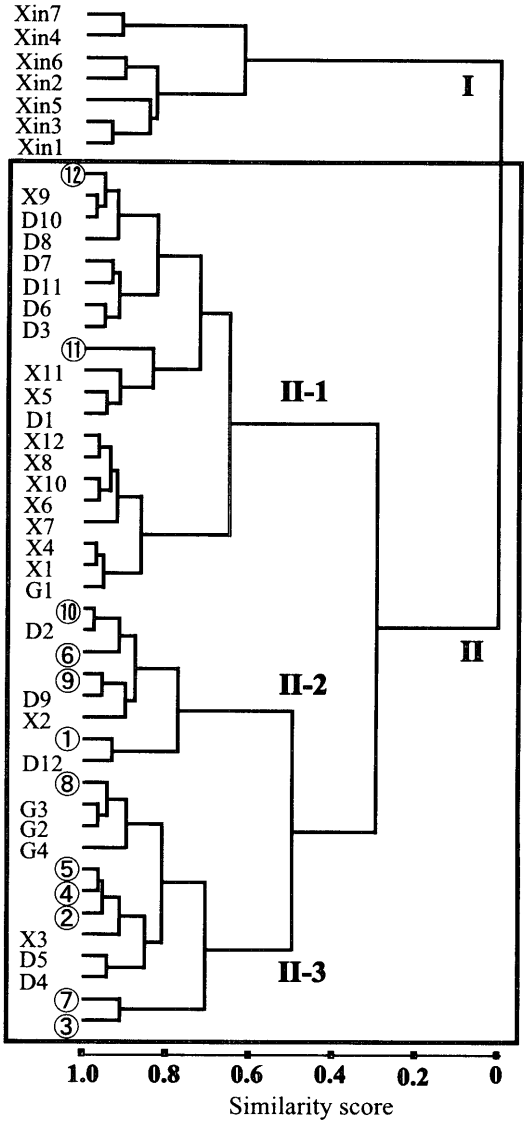

Fig. 6. Hierarchical Cluster analysis (HCA) of 4 kinds of Gancao and *G. uralensis* roots on the basis of 7 HPLC-7-peak-area data.
An HCA examination based on autoscale Euclidian distance and incremental linkage method was carried out on the basis of HPLC-7-peak-area data (**A** to **G**) obtained using the same MeOH extracts as used in PCA. The cluster II-2 had more peaks **C** ($0.90\pm0.21\times10^6$) and **F** ($2.38\pm0.36\times10^6$) area scores ($p<0.05$) than II-1 (**C**: $0.70\pm0.22\times10^6$ and **F**: $1.53\pm0.42\times10^6$) and II-3 (**C**: $0.39\pm0.14\times10^6$ and **F**: $1.96\pm0.39\times10^6$).

JP XIV standard analysis of Daitou-Gancao (Table II)

As shown in Table II, Daitou-Gancao conforms to the 4 standards in addition to the GL-content prescribed in the JP XIV. This suggests that Daitou-Gancao will become an additional resource of medicinal Gancao.

Pharmacological comparison of Daitou-Gancao with Dongbei-Gancao (Fig. 7)

The anti-allergic effect of Daitou-Gancao on the IgE-mediated triphasic skin reaction in mice[4] was

Table II Quality profiles of Daitou-Gancao in relation to JP XIV standard

	GL-content (%)	EtOH-ext. content (%)	Loss on drying (%)	Total ash (%)	Acid-insoluble ash (%)
Daitou-Gancao	6.85±0.47	40.3±1.6	10.7±1.0	4.4±0.3	0.6±0.1
JP XIV standard	2.5 or more	25.0 or more	12.0 or less	7.0 or less	2.0 or less

Each value represents the mean ± S.D. (n= 60 prepared from 12 lots of about 400 roots). The same Daitou-Gancao powder as used in the HPLC profile examination was measured according to the procedure described in the JP XIV.

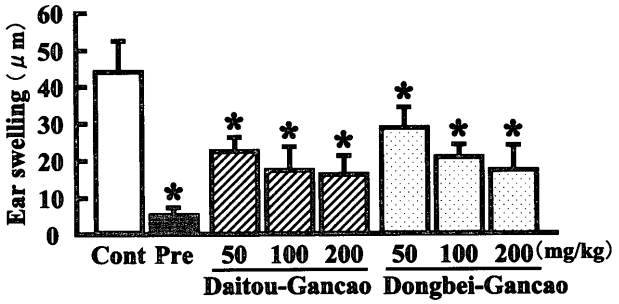

Fig. 7. Effect of Daitou- and Dongbei-Gancao on the very late phase reaction (vLPR) in IgE-mediated triphasic skin reaction in mice.
Each value represents the mean ± S.D. (n= 5).
Cont, Control; Pre, predonisolone-21 acetate (10 mg/kg) were given i.p. 2 h before and 4 to 6 days after DNFB challenge). Gancao extracts (50, 100, 200 mg/kg) were given p.o. 2 h before and 2 to 6 days after the DNFB challenge.
GL-content in water extract (%), Daitou-Gancao (5.65±0.06) ; Dongbei-Gancao (5.95±0.05).
*, $p<0.05$ vs control. (by Mann-Whitney's remove apostrpphe U-test)

Table III Pharmacokinetic parameters of GA after oral administration of Daiou- and Dongbei-Gancao extracts to rats

Parameters	Daitou-Gancao	Dongbei-Gancao
C_{max} (μg / ml)	0.78±0.42	0.63±0.50
T_{max} (h)	9.50±1.22	13.17±8.61
$AUC_{0\text{-}24h}$ (μg h / ml)	8.23±5.18	6.76±3.89

Each extract equivalent to 45 mg/kg of GL was administered to rats. (n=6)
There were no significant differences in the value (mean ± S.D.) of each parameter between the two Gancao.
The $AUC_{0\text{-}24h}$ of GA was calculated by using the trapezoidal rule. The C_{max} and T_{max} of GA were determined by assessing the actual GA levels in the plasma measured using the same HPLC conditions.[5]

compared with that of Dongbei-Gancao. The present triphasic responses contain the immediate phase response (IPR), late phase response (LPR) and very late phase response (vLPR) peaking at 1 h, 24 h and 7 or 8 day after the antigen challenge, respectively. Since the vLPR with massive eosinophil infiltration may be associated with chronic allergic skin reactions in humans,[4] we focused our attention on the efficacy of Daitou- and Dongbei-Gancao against the vLPR. As shown in Fig. 7, oral administration of two Gancao extracts inhibited significantly and dose-dependently the vLPR (ear swelling 7 days after the challenge). The inhibitory potency of Daitou-Gancao was slightly greater than that of Dongbei-Gancao, but there was no significant difference between them. The present results indicate that Daitou-Gancao shows almost an equal anti-allergic effect to medicinal Dongbei-Gancao.

The inhibitory effect of the two Gancao on the IgE-mediated triphasic skin reaction is comparable with that of some traditional Chinese formulations containing Gancao such as Xiao-Qinglong-Tang (Sho-seiryu-to, 小青竜湯), Xiaofeng-San (Shofu-san, 消風散) and Taohe-Chengqi-Tang (Tokaku-joki-to, 桃核承気湯)[17] and Gancao.[9,17] The present studies in the animal model may be associated with the therapeutic usefulness of Gancao-containing formulations, Taohe-Chengqi-Tang[18] and Baihu-Tang (Byakko-to, 白虎湯)[19] in clinical trials for atopic dermatitis.

Biopharmaceutical comparison of Daitou-Gancao with Dongbei-Gancao (Table III)

The biopharmaceutical properties of Daitou-Gancao were compared with those of Dongbei-Gancao, the pharmacokinetics of GA, but not GL, because orally taken Gancao extracts containing GL is biotransformed into an active metabolite GA, which is absorbed into the blood.[20] Therefore, pharamacokinetic parameters were evaluated from the area under the mean concentration of GA versus time curve from zero to 24 h ($AUC_{0\text{-}24h}$), maximum plasma concentration of GA (C_{max}) and the time to reach C_{max} (T_{max}). As shown in Table III, there was no significant difference in the value of C_{max}, T_{max} and $AUC_{0\text{-}24h}$ for GA between the two Gancao. The $AUC_{0\text{-}24h}$ from Daitou-Gancao was slightly greater than that from Dongbei-Gancao, but there was no significant difference between them. The present GA pharmacokinetics data suggest that GL in Daitou-Gancao extract had a similar bioavailability to that in Dongbei-Gancao.

In summary, the purpose of this research was to clarify the feasibility of medicinal use of Daitou-Gancao, which is collected in the confined regions of Gansu and Shanxi provinces of China and a newly imported Chinese Glycyrrhizae Radix. In this regard, to begin with, the botanical origin of Daitou-Gancao collected in the Gansu and Shanxi provinces of China was identified as *G. uralensis* FISCH. by on-the-spot investigations (Figs. 1 and 2). Second, the chemical ingredients of Daitou-Gancao may not differ between medicinal Dongbei- and Xibei-Gancao but are different from those of Xinjiang-Gancao as determined by examining their HPLC fingerprints (Fig. 3), PCA (Fig. 5) and HCA (Fig. 6). Third, Daitou-Gancao fits the five standards prescribed in the JP XIV (Table II). Fourth, it was proved that Daitou-Gancao has almost the same anti-allergic effect on the IgE-mediated triphasic skin reaction as medicinal Dongbei-Gancao (Fig. 7). Finally, it became clear that Daitou-Gancao shows a similar bio-equivalency to Dongbei-Gancao by examining plasma concentration-time profiles and three pharmacokinetic parameters of GA in rats after oral administration of both Gancao extracts (Table III). The present pharmaceutical findings suggest that a newly imported Daitou-Gancao might be appropriate for use as an additional source of medicinal Gancao conforming to the JP XIV.

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和文抄録

1997年以降に中国から輸入されている帯頭甘草（Daitou-Gancao：中国市場の名称）の化学的薬理薬剤学的な特性を評価した。まず，帯頭甘草の基原は，日本薬局方（JP XIV）に適合する *Glycyrrhiza uralensis* FISCH.であることを産地（陝西省と甘粛省）調査から確認した。次に帯頭甘草の glycyrrhizin（GL）を含む HPLC fingerprint は，*G. uralensis* の根と薬用の東北甘草（Dongbei-Gancao）や西北甘草（Xibei-Gancao）に類似し，JP XIV に適合しない新疆甘草（Xinjiang-Gancao）と異なることを明らかにした。米国 FDA は植物由来製品を fingerprint で規格評価することを推奨している。この方法の普及を目指して，fingerprint を bar code に簡略表示することを試みた。なお HPLC profile の定性的な判定結果を principal component analysis（PCA）や hierarchical cluster analysis（HCA）によって客観的に裏づけした。また帯頭甘草が JP XIV の 5 種類の規格試験に適合することも明らかにした。さらに帯頭甘草の抗アレルギー作用（IgE 介在性のマウス耳介腫脹反応）と glycyrrhetic acid（GL の代謝物）の動態は，薬用の東北甘草と同等であることを薬理薬剤学的に検証した。

今回の研究によって，帯頭甘草（陝西省と甘粛省の特定地域に野生する *G. uralensis* の根から調製される甘草）が JP XIV に適合し薬用資源に成り得ることが明らかになった。本論文は中国における産地や流通経路の変動に伴って新たに輸入されてきた帯頭甘草の規格を，含有成分の比較だけでなく薬理薬剤学的にも検討した点に特徴がある。

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