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Field study and pharmaceutical evaluation of *Glycyrrhiza uralensis* roots cultivated in China

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Medicinal licorice (underground parts of *Glycyrrhiza* plants) is an important crude drug prescribed in many traditional Chinese formulations. Recently in China, the collection of wild *Glycyrrhiza* plants was restricted in 1984 because it promotes desertification. With the shortage of licorice due to this restriction, there has been a demand for the cultivation of *Glycyrrhiza* plants.

In the former half of this review, we summarized the results of our survey of the cultivation/production state of *G. uralensis* in China that was performed from 1998 to 2001. In China, the cultivation of *G. uralensis* was initiated in the early 1990s. However, the glycyrrhizin (GL) content of the cultivated roots (mainly 3-year-old roots) we collected in our survey in China did not fulfill the standard (≥2.5%) of the Japanese Pharmacopoeia (JP XIV). Such roots cannot be used for medicine in Japan.

In the latter half of this review, the results of our experiments of *G. uralensis* cultivation in eastern Neimenggu autonomy in China were summarized. We performed cultivation from seeds of *G. uralensis*, and taproots were transplanted the next year. As a result, adventitious roots collected in autumn in the 4th year after seeding conformed to the JP XIV standard for GL content. This study was the first to clarify the appropriateness of *G. uralensis* roots cultivated in China as a resource of medicinal licorice. The anti-allergic action of the cultivated roots and their GL bioavailability after oral administration were similar to those of existing medicinal licorice prepared from wild *G. uralensis* in China. These results suggest that cultivated *G. uralensis* roots can compensate for the insufficient licorice resources.

This study not only aims at the development of licorice resources and the assessment of their standards but is also a phyto-remediation study on the global environment using cultivated plants.

Key words *Glycyrrhiza uralensis,* cultivation, glycyrrhizin, HPLC, licorice, anti-allergic effect.

Abbreviations AUC, the area under the concentration-time curve; C_{max}, maximum plasma concentration; Dongbei-Gancao, Tohoku-Kanzo: 東北甘草; GA, glycyrrhetic acid; GAP-MP, Good Agricultural Practice for Medicinal Plants in Japan; GL, glycyrrhizin; JPXIV, The Japanese Pharmacopoeia XIV edition; Neimenggu, 内蒙古自治区; PCA, principal component analysis; ShanghanLun, Shokanron, 『傷寒論』; Xibei-Gancao, Seihoku-Kanzo: 西北甘草.

1. Introduction

Sufficient resources for crude drugs produced in China, the assessment of their standards, and their stable supply are important issues at the basis of traditional Chinese medical treatment (Kampo-remedy in Japanese). Among crude drugs produced in China, licorice (Glycyrrhizae Radix) as well as Ephedrae Herba and Puerariae Radix have been prepared from wild plants. However, since the collection of wild plants has caused destruction of nature, wild plants as a resource have decreased, resulting in restrictions of their collection and a decrease in their output. Districts producing Bupleuri Radix, Scutellariae Radix, and Poria in China have already changed, and cultivated products have been increasing.

There is a possibility that crude drugs prepared from cultivated plants differ in characteristics from conventional drugs prepared from wild resources. Since cultivated plants are new drug resources, whether or not crude drugs prepared from cultivated plants conform to the standards of official compendiums such as the Japanese Pharmacopoeia (JP XIV) should be determined. After the confirmation of the conformity, the crude drugs prepared from cultivated plants should be compared with those prepared from wild plants pharmacologically and biopharmaceutically.

For licorice, in 1984, the Chinese government restricted the collection of *Glycyrrhiza* plants by people living in regions other than the 3 northern regions (Gansu-province：甘粛省and two autonomies of Neimenggu: 内蒙古自治区 and Ningxia: 寧夏回族自治区), because excessive collection of *Glycyrrhiza* plants induces desertification. In areas where *Glycyrrhiza* plants naturally grow, there are a nature reserve as shown in Fig. 1.

The restrictions of the collection of wild *Glycyrrhiza* plants in China have been tightened, and changes in quality

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Fig. 1. Marker of preservation of *Glycyrrhiza* plants
At Ziniquan (紫泥泉) in Xinjian autonomy (新疆維吾爾自治区). Aug. 2002

due to a decrease in output, changes in producing districts, and cultivation are expected in the future. Therefore, we performed a survey of the cultivation status of *Glycyrrhiza* plants in China. After the survey, a *G. uralensis* cultivation experiment was performed in the eastern region of Neimenggu autonomy.

In this review, the survey data and the standard of cultivated roots[1] were compared with those of medicinal licorice on the Japanese market,[2] and the results of studies on pharmacological and pharmaceutical characteristics[3] were summarized.

If cultivated roots are made fit for practical use, the standard and supply of licorice will be stabilized. In addition, the collection of wild *Glycyrrhiza* plants can be reduced, which may contribute to the conservation of the global environment.

2. Overview of licorice

Licorice is prepared from the underground part (mainly the roots) of *Glycyrrhiza* plants (such as *G. uralensis* Fisch. and *G. glabra* L.). *Glycyrrhiza* plants grow in the east and west regions of the Eurasian Continent, i.e., from Spain to Central Asia, Mongolia, and China (northwest-northeast regions) and have been long used for medicine both in the East and the West.[4,5] The extract of the underground parts of *Glycyrrhiza* plants is also widely used as a natural sweetener for candies and soy sauce,[6] but harm from excessive intake has also been suggested.[7]

Extensive studies on licorice have been performed,[8] and glycyrrhizin (GL) as an active component was developed as a medicament for the treatment of allergic inflammatory diseases and chronic hepatitis and have been used up to the present. Recent studies have shown the inhibitory effects of GL on severe acute respiratory syndrome (SARS)-associated corona-virus.[9]

Licorice (Gancao in Chinese) in China was described as a drug for wounds in a traditional medical book in the 2nd century B.C. that was excavated in the old tomb of Mawang (馬王堆漢墓), and therefore, has a history of medicinal use for more than two thousand years. Gancao is the most frequently used crude drug (64.3%)[10] in an important traditional Chinese medical formulary ShanghanLun (『傷寒論』).[11] Many prescriptions in ShanhanLun include particular combinations of two crude drugs (Glycyrrhizae Radix－Zizyphi Fructus and Glycyrrhizae Radix－Zingiberis Rhizoma),[12] and then various formulations were established using Glycyrrhizae Radix－Cinnamomi Cortex, Glycyrrhizae Radix－Ephedrae Herba, Glycyrrhizae Radix－Paeoniae Radix, and Glycyrrhizae Radix－Rhei Rhizoma as base axes.[13]

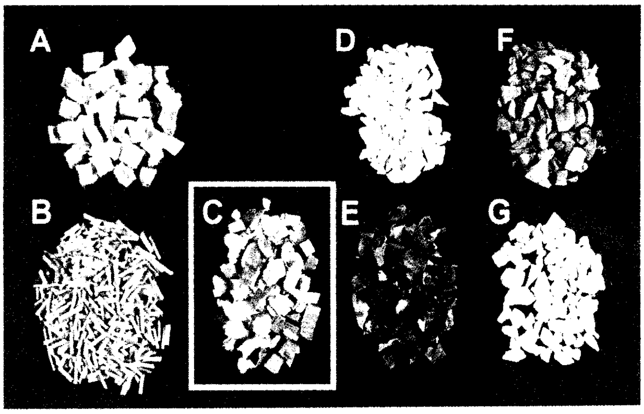

Fig. 2. Seven crude drugs prescribed in Kakkonto (GegenTang)
Kakkonto (葛根湯) is one of the famous Kampo-formulations used in Japanese medical care.
Seven crude drugs prescribed in Kakkonto are of the Japanese Pharmacopocia XIV standard. **A**: Puerariae Radix (4.0 g/day); **B**: Ephedrae Herba (3.0 g/day); **C**: Glycyrrhizae Radix (2.0 g/day); **D**: Zingiberis Rhizoma (2.0 g/day); **E**: Ziziphi Fructus (3.0 g/day); **F**: Cinnamomi Cortex (2.0 g/day); **G**: Paeoniae Radix (2.0 g/day).
Kakkonto-preparation for ethical use contains the freeze-dried extract (approximately 3.75 g/day) prepared from Kakkonto decoction, which is traditionally decocted from the combination (30 g of crude drugs mixture) by 600 ml of water for 40 min.

Licorice (Kanzo in Japanese) contained in the JP XIV[14] has been used in many Kampo-preparations for ethical use (医療用漢方製剤) such as Kokkanto (葛根湯：GegenTang in Chinese: Fig. 2). Medicinal Kanzo used in Japan is mainly produced in China (especially Dongbei-Gancao: 東北甘草).

In Japan, there are three types of Kanzo (Gancao) imported from China. Dongbei-Gancao is light and soft and produced in the northeast region of China, particularly the eastern region of Neimenggu autonomy and Jilin-province (吉林省). Processing is performed, leaving the knot-like swollen part in the root head (Fig. 3). Xibei-Gancao (西北甘草) has hard heavy roots and is produced in Ningxia and the western region of Neimenggu autonomy, and the northwest region of China such as Shanxi- (陝西省) and Gansu-provinces (甘粛省).[15] Xinjian-Gancao (新疆甘草) is also imported as a GL extraction material or food additive, but it does not fulfill the several standards of JP XIV.

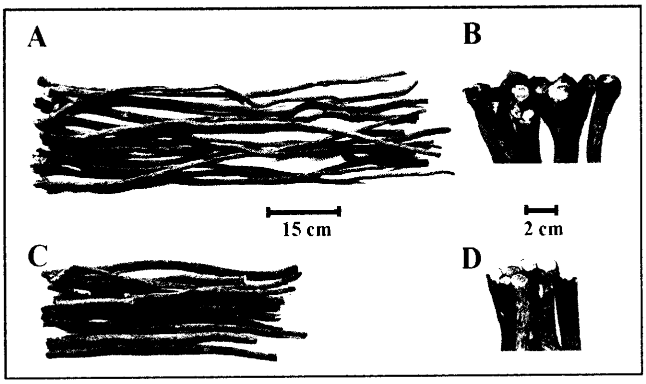

Fig. 3. Dongbei-Gancao and Xibei-Gancao
A: whole shape of Dongbei-Gancao (東北甘草), B: root head of Dongbei-Gancao, C: whole shape of Xibei-Gancao (西北甘草), D: root head of Xibei-Gancao
Average GL content (3.39～**5.16**±**1.00**～7.57%, 109 lots) of Dongbei-Gancao in Japanese drug market between 1986 and 2000 is more than that (2.43～**4.36**±**1.45**～7.99%, 228 lots) of Xibei-Gancao. (p<0.05)
【from Ref. 2)】

3. Field study of cultivation of *G. uralensis* in China

3.1) Outline of cultivation status in China: Medicinal Kanzo used in Japan depends on wild *Glycyrrhiza* plants in China. In 2000, this restriction of the wild *Glycyrrhiza* plants collection was tightened. To compensate for insufficient resources due to collection restrictions, cultivation is indispensable.

Before our research on cultivation in China,[16] we surveyed the status of cultivation of *Glycyrrhiza* plants in China from 1998 to 2001 (Fig. 4).[17] As a result, research on *G. uralensis* cultivation in China was initiated in the 1980s,[18] and practical-scale cultivation was started in the early 1990s in northwest regions (Shanxi-province and the western region of Neimenggu) and in the mid-1990s in the northeast region (Jilin-province and the eastern region of Neimenggu). An interview survey of the distribution of cultivated licorice in 2000 in a crude drug export corporation in Hebei-province (河北省) showed that *G. uralensis* roots cultivated in the northeast region are mainly used in China and partly exported into Korea. However, due to a low GL content, the amount of export to Korea decreased, and the production decreased in the latter half of the 1990s.

3.2) Seeding of *G. uralensis*: The state of cultivation fields in Chifeng (赤峰) in the eastern region of Neimenggu autonomy (Fig. 5) is described in the following (survey period: Aug. 2000-Aug. 2003). The cultivation method was seed propagation, and seeding was performed from April-early May (Fig. 6). For cultivation, seeds (about 3 mm in long axis: Fig. 7) collected from wild *G. uralensis* were

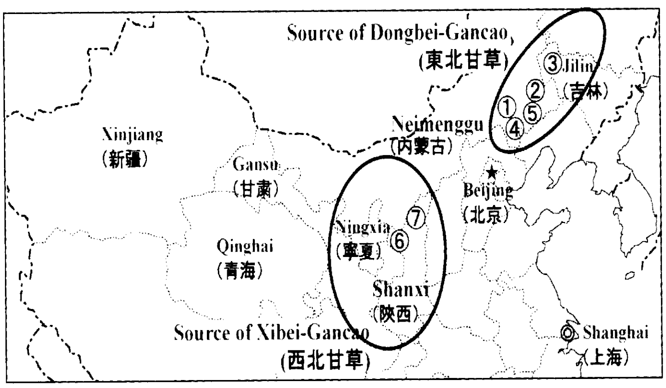

Fig. 4. Field study of cultivation spot of *Glycyrrhiza uralensis* and major sources of Dongbei-Gancao and Xibei-Gancao
Area of source of Dingbei-Gancao
1) Wengniuteqi in Neimenggu (内蒙古自治区翁牛特旗) (May. 2000)
2) Tongliao in Neimenggu (内蒙古自治区通遼市) (May, Aug, Oct. 2000)
3) Tongyu-xian in Jilin province (吉林省通楡縣) (May, Aug. 2000)
4) Yuanbaoshan-qu in Neimenggu (内蒙古自治区元宝山区) (Aug, Oct. 2000)
5) Aohanqi in Neimenggu (内蒙古自治区敖漢旗) (Aug. 2000)
Area of source of Xiebei-Gancao
6) Dingbian-xian in Shanxi province (陝西省定辺縣) (Jul. 2001)
7) Zizhou-xian in Shanxi province (陝西省子洲縣) (Jul. 2001)

Fig. 5. Cultivation field of *G. uralensis* seeds
At Yuanbaoshan-qu in eastern Neimenggu (Aug. 2000)

Fig. 6. Sowing of *G. uralensis* seeds
At Yuanbaoshan-qu in eastern Neimenggu

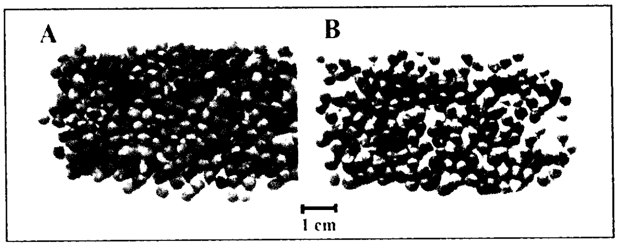

Fig. 7. Seeds of *G. uralensis*.
Seeds collected at Yuanbaoshan-qu in eastern Neimenggu (**A**) and at Yanchi (塩池) in Ningxia autonomy (**B**: 寧夏回族自治区).

commercially available. In some cultivation districts, primary school students collected the seeds of wild *G. uralensis* that grow around cultivation fields during their summer vacation.

Seeds are sometimes coated with a moistening agent or manure, but details were not clarified because of the preservation of confidentiality.

3.3) Transplantation of 2-year-old roots: The difference in the cultivation method between the two regions was the presence or absence of root transplantation (Fig. 8). In the northwest region, no transplantation was performed after seeding. In the northeast region, in spring in the year after seeding, taproots were cut to 25-30 cm and transplanted, being inclined with the root head about 5-10 cm underground and the root tip about 12-20 cm underground, at about 10-cm intervals (Fig. 9). This transplantation with inclination (about 10°) was because vertical growth of taproots makes harvesting difficult.

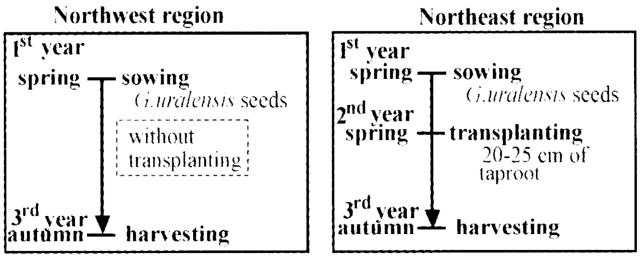

Fig. 8. General cultivation conditions of *G. uralensis* in northwest and northeast regions of China
It is common in the northeast region Dongbei-Gancao producing districts to sow the seeds in spring, and to dig up and transplant the seedlings in the next spring. Aohan-qi, Wengniute-qi and Yuanbaoshan-qu in eastern Neimenggu, Tongyu-xian in Jilin province.
In some northeast regions (some areas of Yuanbaoshan-qu in eastern Neimenggu and Tongyu in Jilin province), the seeds of *G. uralensis* are sowed in spring and the seedlings' roots are transplanted in the autumn of the same year and cultivated for 3 years.

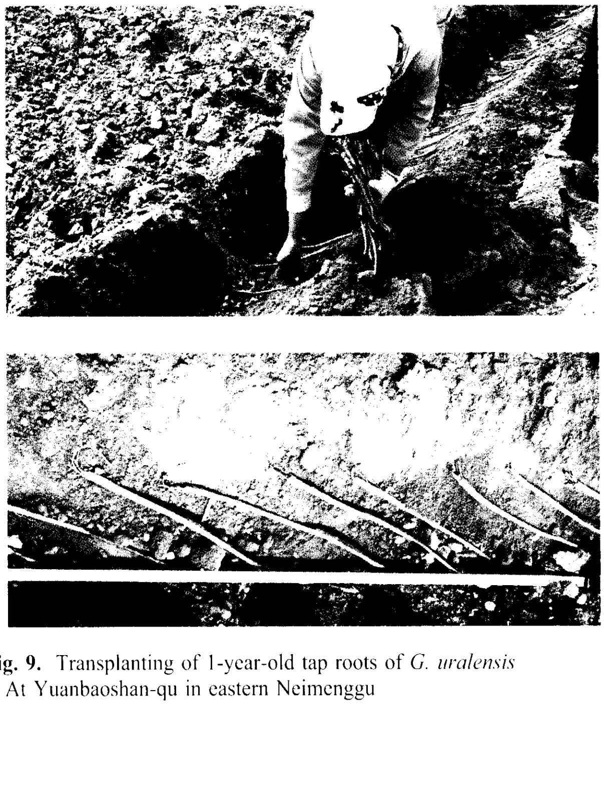

Fig. 9. Transplanting of 1-year-old tap roots of *G. uralensis*
At Yuanbaoshan-qu in eastern Neimenggu

3.4) Identification of cultivated plants: The aboveground part of 2-year-old plants that had been transplanted in spring grew to 30-45 cm in summer, and roots showing good growth had a length of 40 cm and a root head diameter of 1.5 cm (Fig. 10). The aboveground part in August 2 years after transplantation (3-year-old plants) grew to 80-140 cm. Since seed selection was not performed, there were marked individual differences in growth and the flowering time.

Cultivated plants had a longer aboveground length than wild plants that grew in areas around the cultivation areas. Some plants flowered when they were 3 years old. These 3-year-old plants were confirmed to be *G. uralensis* by comparing the characteristic morphology of their fruits (Fig. 11) with a plant taxonomical report.[19]

Fig. 10. 2-Year-old of *G. uralensis*
At Yuanbaoshan-qu in eastern Neimenggu (Aug.2000)

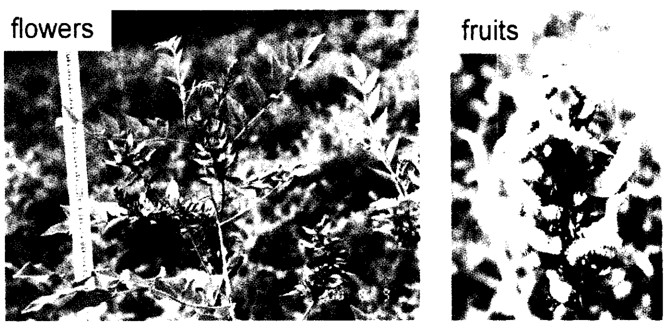

Fig. 11. Flowers and fruits of 3-year-old *G. uralensis*
At Yuanbaoshan-qu in eastern Neimenggu (Aug.2000)

3.5) Digging and preparations of 3-year-old roots: The harvest time was October in the 3rd year after seeding (2 years after transplantation), and roots were collected by digging using tractors (Fig. 12). The obtained plants were manually divided into the aboveground parts, stolons, and roots. The roots were classified into thick (root head, 13-20 mm) and thin (6-13 mm) (Fig. 13). These cultivated roots were stored in warehouses nearby (Fig. 14) and shipped.

3.6) GL content of cultivated roots: The GL content of the 3-year-old cultivated roots shown in Fig. 14 was measured by the JP XIV methods. The GL content was about 1.03-1.62%, and did not conform to the JP XIV standard (≥2.5%). The GL content of cultivated roots collected in other districts were also measured, but none of them fulfilled the JP XIV standard (5-year-old roots: 2.41±0.87%; 6-year-old roots: 2.48±1.08%). Though the GL content in 11-year-old roots collected in the northwest region was 2.58 ±0.68%, 11 years cultivation is not practical. A reference in China[18] showed a GL content of more than 8% in 3- or 4-year-old *G. uralensis* roots cultivated in the northwest region. However, since there was no description of quantification methods in this reference, comparison of reported data with our results could not be made.

The GL content of 2- or 3-year-old roots cultivated and transplanted in the northeast region did not conform to the JP XIV standard, but there is a possibility that the GL content increases with cultivation years (2-year-old roots: 1.35 ±0.58%; 3-year-old roots: 1.61±0.45%). An experiment of *G. uralensis* cultivation in Japan also showed a GL content of ≥2.5% in 3-year-old roots.[20] Therefore, the GL content in roots cultivated for 3 years or less may not reach the JP XIV standard.

4. Cultivation and evaluation study of *G. uralensis* in Neimenggu

4.1) Outline of cultivation study in Neimenggu: Based on the results of the survey in cultivation districts in China, experiments to obtain cultivated roots fulfilling the JP XIV standard were performed in a field (about 33 a) in

A

B

C

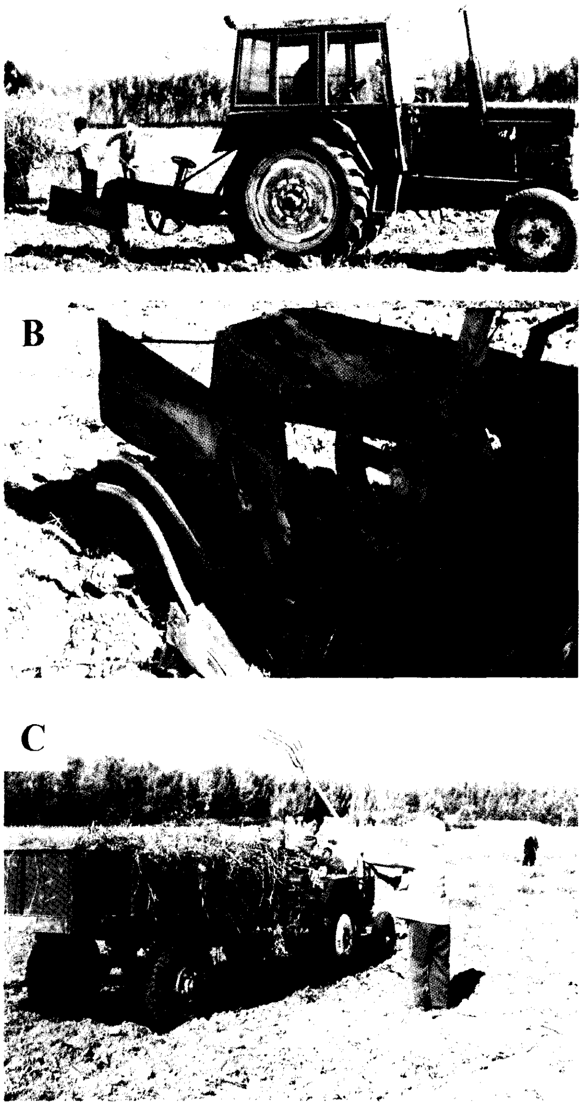

Fig. 12. Digging and gathering of 3-year-old cultivated G. *uralensis* roots
At Yuanbaoshan-qu in eastern Neimenggu (Oct.2000)
A: Tractor with plow; **B**: Plow (expanded); **C**: Gathering the roots

Fig. 13. Preparation of 3-year-old cultivated *G. uralensis* roots
At Yuanbaoshan-qu in eastern Neimenggu (Oct.2000)

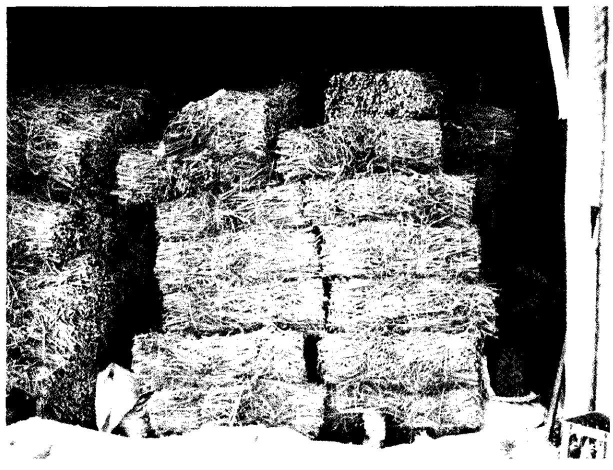

Fig. 14. 3-year-old Cultivated *G. uralensis* roots
At Tongyu-xian in Jilin province (Oct.2000)

Yuanbasoshan-qu (元宝山区) in the suburbs of Chifeng in the eastern region of Neimenggu, about 350 km northeast of Beijing (Fig. 15). The annual rainfall in this area is about 350 mm, and the mean temperature is 23.5℃ (mean in July) — -11.5℃ (mean in January).

This region was the main district producing Dongbei-Gancao, and wild *G. uralensis* grew around the cultivation lands. Wild *G. uralensis* was collected by farmers in the neighborhood from May to October (Fig. 16). This indicates that the collection period of wild *G. uralensis* is not consistent. In this region, *G. uralensis* had been cultivated since 1996. Around the cultivation field, *Isatis indigotica* as a resource for Isatidis Radix and *Astragalus membranaceous* as a resource for Astragali Radix were also cultivated.

The cultivation experiment field was divided into 5 sections, and cultivation was performed for 7 years (Fig. 17). Samples were annually collected (Fig. 18). For cultivation, seeds collected from wild *G. uralensis* in Baotou (包頭) in western Neimenggu were purchased in a market in Anguo (安国) in Hebei province. The cultivated plants were confirmed to be *G. uralensis* based on the characteristic morphology of fruits obtained from July to August from the 3rd to 5th year.

In recent years, the methods of medicinal plant cultivation and the quality assessment of their crude drugs (Good

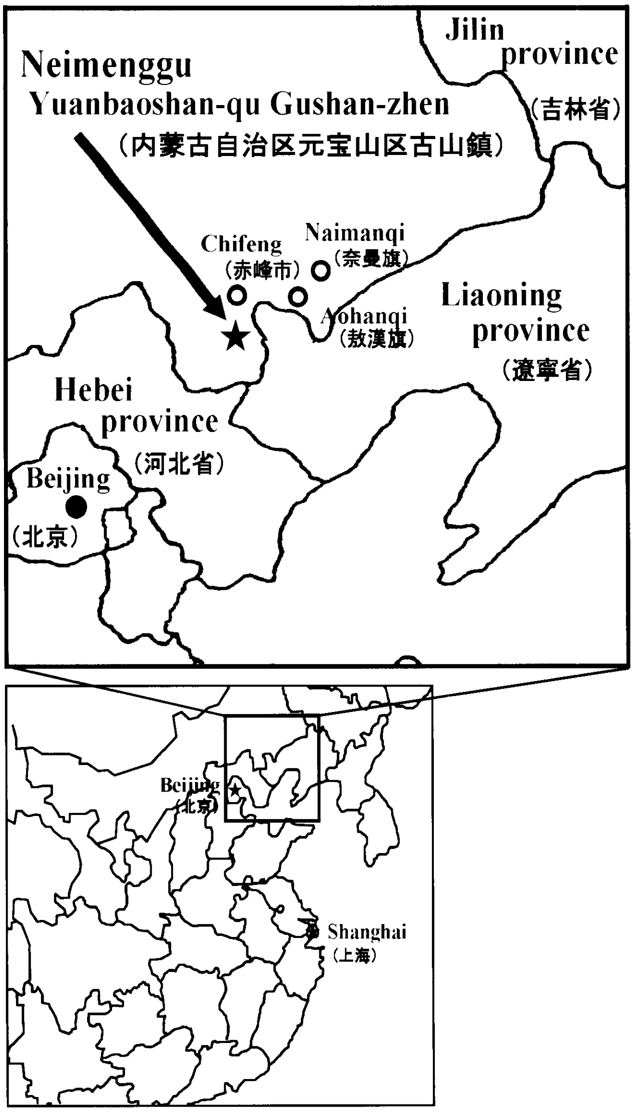

Fig. 15. Cultivation study field in eastern Neimenggu
The cultivation filed is located in Yuanbaoshan-qu, in the suburbs of Chifeng (赤峰), eastern Neimenggu in China, 118° 97'E longitude and 42° 27' N latitude.
Naiman-qi, Aohan-qi and Chifeng are the major source of Dongbei-Gancao.

Fig. 16. Collection of wild *G. uralensis* by farmers
At Wengniute-qi in eastern Neimenggu (Aug.2000)

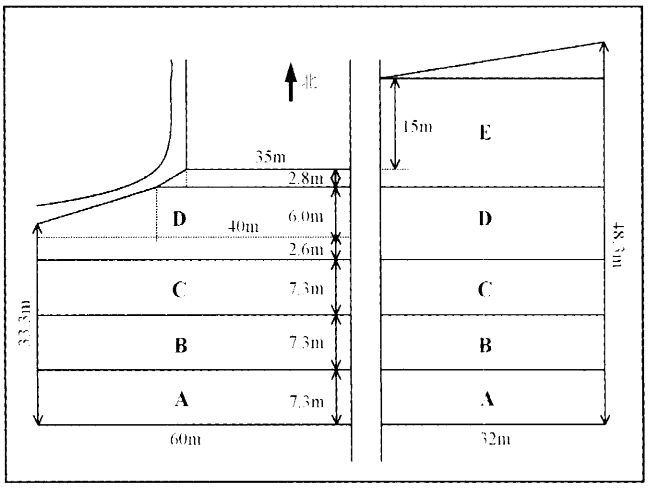

Fig. 17. Division of cultivation study field in eastern Neimenggu
A-E: The division sampling at 3-, 4-, 5-, 6-, and 7-year-old roots
Seeds were collected in 1997 from wild *G. uralensis* growing in Baotou (包頭) in western Neimenggu. The 3-year-old cultivated plants come into blossom in July to August.

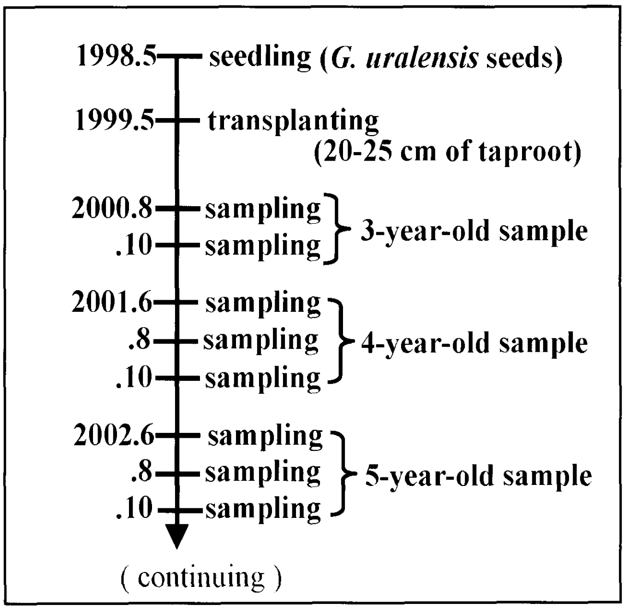

Fig. 18. Sampling schedule of *G. uralensis* roots from the cultivation study field in eastern Neimenggu

Agricultural Practice for Medicinal Plants in Japan: GAP-MP) have been evaluated. Since this study was initiated in 1998, the control described in GAP was not performed. In the future, cultivation in conformity with GAP and GAP-MP such as the control of heavy metals and agricultural chemicals may become necessary.

4.2) Growth of roots cultivated in Neimenggu[1]: The underground part of cultivated *G. uralensis* consisted of the taproot observed at the time of transplantation and adventitious roots and stolons that newly grew from the tip of the taproot after transplantation (Fig. 19). The diameter of the base of the taproot in October in 4-year-old roots was 1.43 ±0.25 cm (n = 6), which corresponded to No. 2 (≥1.0 cm) in the export standard of Dongbei-Gancao on the market. The length of the taproot in 4-year-old and 5-year-old plants was 20-25 cm, which was similar to the length at the time of transplantation. Though we expected transplanted taproots to grow thick and long, they did not extend while adventitious roots grew from the cut end. This suggested that inclination at the time of transplantation is not necessary.

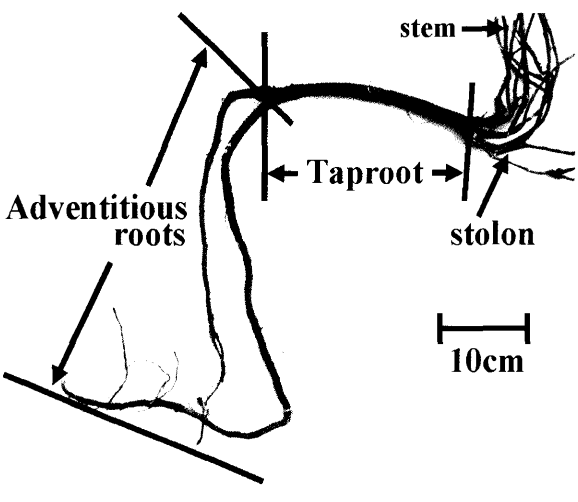

Fig. 19. 4-year-old underground parts of *G. uralensis* roots cultivated in eastern Neimenggu

The diameter at the base of adventitious roots was 0.81 ±0.16 cm (n = 6), which corresponded to No. 3 (≥0.5 cm). The length of adventitious roots became more than 50 cm in 4-year-old or older roots. The number of adventitious roots did not differ among cultivation years (3-year-old roots, 2.7 ±1.0; 4-year-old roots, 2.6±1.0; and 5-year-old roots, 2.6 ±1.0; n = 18). The root weight increased in October in both 4-year-old and 5-year-old roots, but no increase was observed from October in 4-year-old roots (71.9±41.4 g, n = 18) to October in 5-year-old roots (67.5±20.0 g, n = 18) (Fig. 20).

The ratio of the taproot to adventitious roots in October in 4-year-old roots was almost 3 : 4, and their yields as dry substance were 21 kg/a and 29 kg/a, respectively. In terms of only yield, harvest in autumn 3.5 years after seeding (October in 4-year-old roots) may be appropriate.

4.3) GL contents of roots cultivated in Neimenggu[1]: To clarify the collection time and cultivation years to obtain the GL content that conforms to the JP XIV standard, seasonal and annual changes in the GL content (%) were evaluated. The GL content in cultivated roots 2.5 years after seeding (3-year-old roots) did not reach the JP XIV standard (entire part of 3-year-old roots in October, 1.69±0.55%; taproot, 1.37±0.44%; adventitious root, 2.24±0.62%; n = 7).

The GL content in adventitious roots after October in 4-year-old roots conformed to the JP XIV standard, the maximum value being 4.14±0.79% (June, 5-year-old roots) (Fig. 21). However, the GL content of taproots was 2.29±0.58% even at the maximum (June, 5-year-old roots; n = 18), and no sample older than 4-year-old tap roots reached the JP XIV standard. Therefore, we decided to use adventitious

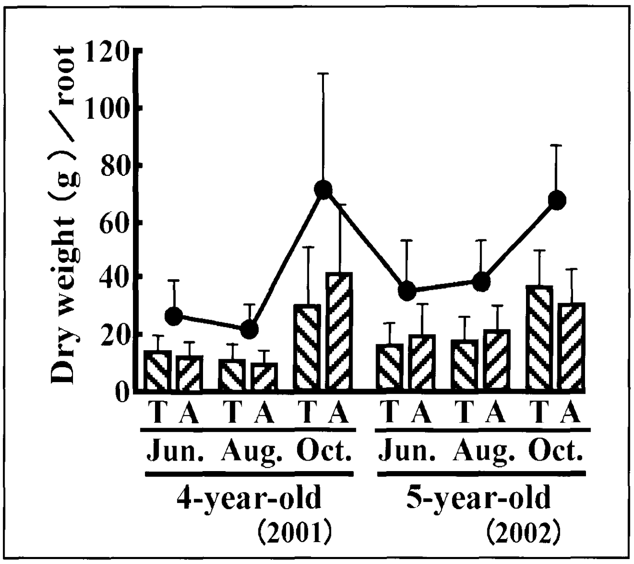

Fig. 20. Dry weight of taproot (T) and adventitious root (A) of *G. uralensis* roots cultivated in eastern Neimenggu
Each value represents the mean ± S.D. (n= 18).
Line graph represents dry weight per whole root.
【Modified from ref. 1)】

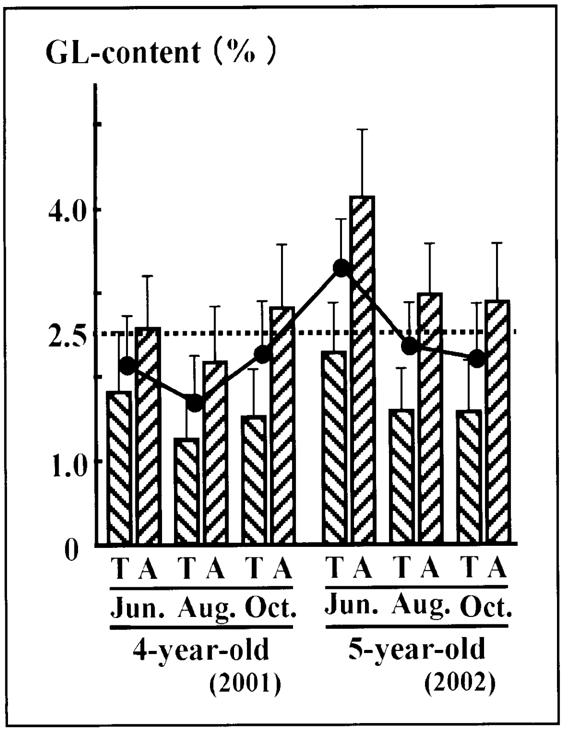

Fig. 21. GL-content (%) of taproot (T) and adventitious root (A) of *G. uralensis* roots cultivated in eastern Neimenggu
Each value represents the mean±S.D. (n= 18).
Dotted line at 2.5% represents the lower limit of JP XIV standard of GL-content.
Line graph represents GL-content (%) per whole root.
【Modified from ref. 1)】

roots that grow from the tip of the taproot as resources of medicinal licorice.

In June in 5-year-old roots, the GL content in the entire root including the taproot fulfilled the JP XIV standard (3.28±0.60%: maximum value in adventitious roots during the evaluation period, 4.14±0.79%). However, the GL content per root (1.17±0.63 g/root) was lower than that in October in 4-year-old roots (1.61±0.93 g/root), and taproots with GL contents (2.29±0.58%) that did not conform to the JP XIV standard. These results showed that adventitious roots that conform to the JP XIV standard of the GL content can be obtained in autumn or later (October, 4-year-old roots) 3.5 years after seeding in spring (May).

The GL contents of taproots and stolons obtained at the harvest of adventitious roots (October, 4-year-old roots) did not fulfill the JP XIV standard (taproot, 1.52±0.57%, n = 18; stolon, 2.36±0.85%, n = 6). However, these parts can be effectively utilized as materials for GL extraction.

4.4) GL contents of cultivated roots with or without flowers: About 10% of cultivated *G. uralensis* plants flowered in summer when they were 3 years old. This percentage of plants with flowers did not differ between 4-year-old plants and 5-year-old plants. The GL content of adventitious roots of plants with flowers (2.73±0.77%, n = 6) collected in August from 4-year-old plants was significantly higher ($p < 0.05$) than that of plants without flowers (2.00 ±0.32%, n = 6). These results suggest that the GL content can be increased by increasing the number of plants that flower in the 3 years. The selecting seeds that flower in the 3rd year is the subject for a future cultivating study.

4.5) HPLC-profile of roots cultivated in Neimenggu[3)]: For multi-component crude drugs, the Food and Drug Administration (FDA) recommends the control of the standards of their components including the active component by methods such as HPLC fingerprint analysis.

As shown in Fig. 22, the major peaks of 4-year-old adventitious roots were peak F (GL) and peak B (liquiritin) at a detection wavelength of 254 nm and peak C (isoliquiritin apioside) and peak D (isoliquiritin) at 350 nm. This HPLC fingerprint was similar to the fingerprints of Dongbei-Gancao and Xibei-Gancao for medicinal use prepared from

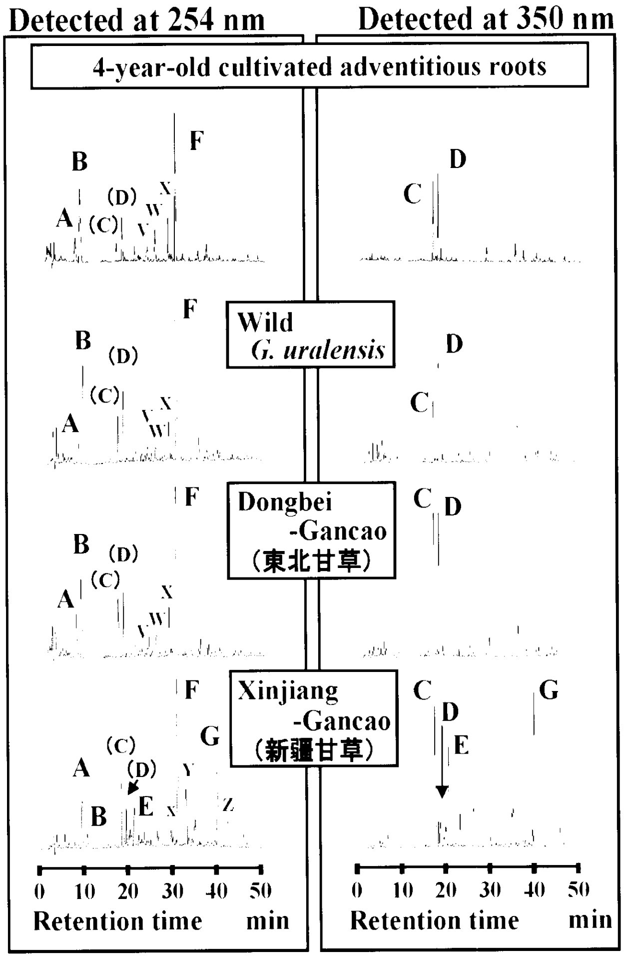

Fig. 22. HPLC fingerprints of cultivated *G. uralensis* roots and comparative samples
For HPLC fingerprint analysis, MeOH extracts prepared from adventitious roots of 4-year-old cultivated G. uralensis were used. As comparative samples, MeOH extracts prepared from Dongbei-Gancao, Xinjiang-Gancao and wild *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.
【Modified from ref. 3)】

G. uralensis roots and wild *G. uralensis* roots collected around the cultivation experiment area. The HPLC fingerprints obtained in this experiment closely resembled previously reported chromatograms.[21)]

On the other hand, the HPLC fingerprint of Xinjiang-Gancao showed characteristic peaks such as peak G (lichochalcone A) that were not observed in *G. uralensis* roots. HPLC fingerprint analysis showed that the composition of 4-year-old indefinite roots resembles that of medicinal licorice and can be distinguished from that of Xinjiang-Gancao that does not conform to the JP XIV standard.[22)]

For objective evaluation of this qualitative profile, multivariate analysis was performed using the areas of the 7 peaks (peaks A-G) on HPLC fingerprints as variables. Principal component analysis (PCA) showed that the distribution of cultivated *G. uralensis* roots is similar to that of medicinal licorice or wild *G. uralensis* roots but definitely differs from that of Xinjiang-Gancao (Fig. 23). Therefore, quantification of the results of HPLC fingerprint analysis by PCA showed no differences in components between cultivated adventitious roots and medicinal licorice.

Thus, adventitious roots dug up in October in the 4th year after seeding (Fig. 24) conformed to the JP XIV standard including the GL content. This study is the first to show the conformity of *G. uralensis* roots cultivated in China with the medicinal use standard in Japan. The GL content of 4-year-old cultivated roots (2.81 ± 0.76%) was not satisfactory compared with the mean value of medicinal licorice on the market in Japan during a 15-year period (mean maximum value, 6.89 ± 0.92%; mean minimum value, 2.82 ± 0.24%).[2)]

We have been performing further studies on the selection of seeds, the presence or absence and time of transplantation, and cultivation conditions.

4.6) Anti-allergic effects of 4-year-old roots cultivated in Neimenggu[3)]: When the usefulness of multi-component crude drugs is evaluated, it is desirable to evaluate pharmacological effects after oral administration. The pharmacological action (anti-allergic action) of adventitious roots collected in October in the 4th year was compared with that of existing medicinal licorice (Dongbei-Gancao).

Kanzo is contained in Kampo-preparations used for the treatment of allergic inflammatory disease, peptic ulcer, and hepatopathy. In particular, for atopic dermatitis (allergic dermatitis), Kampo-preparations containing Kanzo such as Byakkokaninjinto (白虎加人参湯),[23)] Shofusan (消風散),[24)] and Tokakujokito (桃核承気湯)[25)] are clinically used. The anti-allergic effects of Byakkokaninjinto was shown[26)] in IgE-mediated trophasic skin reactions in mice.[27)] In this pathological model, an immediate (first) phase response (IPR) was observed 1 hour after induction of responses, auricular swelling as a late (second) phase response (LPR) after 1 day, and an inflammatory phase as a very late (third) phase response (vLPR) with a peak after 6-8 days. In the third phase (vLPR), marked eosinophil infiltration was observed in the swollen auricles, indicating the involvement of T cells. Therefore, this phase is considered to correspond to a human allergic dermatitis model.[27)]

Among crude drugs prescribed in Byakkokaninjinto, Kanzo has been shown to be involved in anti-allergic action.[26)] Therefore, the pharmacological characteristics of 4-year-old cultivated adventitious roots were compared with those of medicinal Dongbei-Gancao using this model. Anti-allergic effects were similar between both extracts (Fig. 25).

4.7) GL-bioavailability from 4-year-old roots cultivated in Neimenggu[3)]: For the evaluation of new medicinal resource candidates, pharmacokinetic information of the active component is also necessary. In particular, since crude drugs contain many components affecting the absorption of the active component, biopharmaceutical analysis is important. Orally administered GL is converted to glycyrrhetic acid (GA) by intestinal bacteria and absorbed into the plasma.[28)] Since GA is an active metabolite with anti-allergic effects,[29)] the bioavailability of GL can be

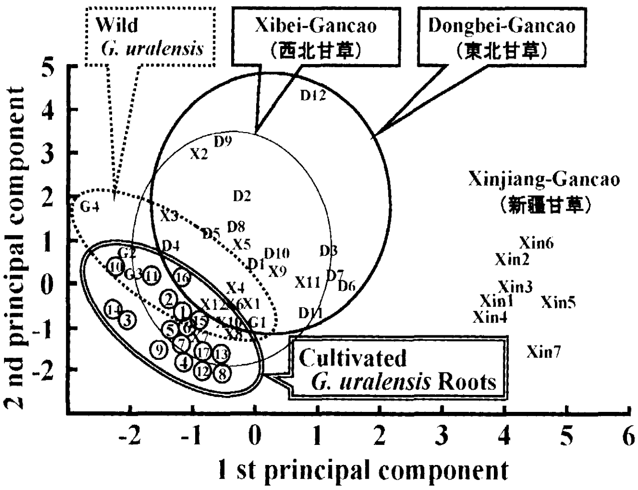

Fig. 23. Scatter diagram of principal component score on the basis of HPLC-7-peak area data

A principal component analysis was carried out on the basis of HPLC-7-peak-area data (**A** to **G** of Fig. 22) obtained using the same MeOH extracts prepared from cultivated roots, Dongbei-Gancao, Xibei-Gancao, Xinjiang-Gancao and wild *G. uralensis* roots, respectively.

①-⑥:4-year-old adventitious roots (Oct.2001); ⑦-⑨:4-year-old taproots (Oct.2001); ⑩-⑪:5-year-old adventitious roots (Jun.2002); ⑫-⑬:5-year-old taproots (Jun.2002); ⑭-⑯:5-year-old adventitious roots (Oct.2002); ⑰:5-year-old taproot (Oct.2002);

D1-D12, Dongbei-Gancao (D1, Neimenggu in 1990; D2-5, Hong Kong market in 1991; D6-8, Neimenggu in 1992; D9, Neimenggu in 1994; D10, Hebei (河北) in 1996; D11, Neimenggu in 1998; D12, Neimenggu in 1999);

X1-X12, Xibei-Gancao (X1, Neimenggu in 1991; X2-3, Qinghai (青海) in 1992; X4, Ningxia in 1993; X5, Ningxia in 1994; X6, Shanxi (陝西) in 1997; X7-8, Ningxia in 1998; X9-12, Ningxia in 2000);

Xin1-Xin7, Xinjiang-Gancao (Xinjiang and Hebei in 2000);

G1-G4, Wild G. uralensis roots (eastern Neimenggu in 2000).

【Modified from ref. 3)】

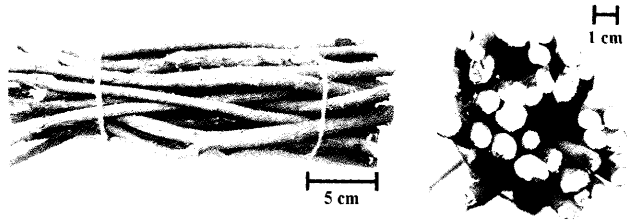

Fig. 24. 4-year-old adventitious roots cultivated in eastern Neimenggu

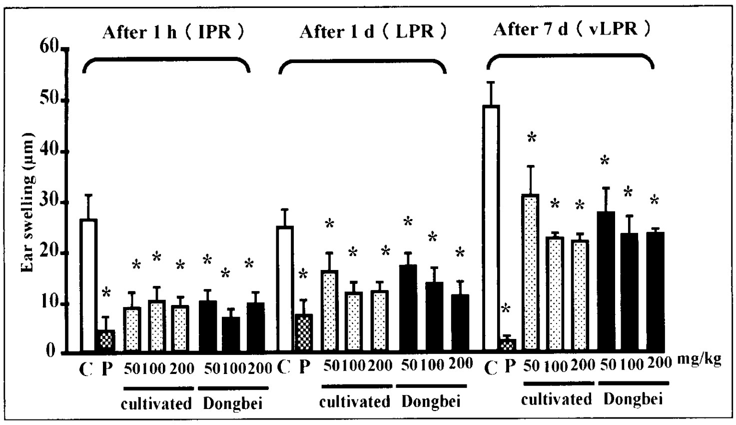

Fig. 25. Effects of the 4-year-old adventitious roots and Domgbei-Gancao on the IgE-mediated triphasic skin reaction in mice
Each value represents the mean ± S.D. (n= 5).
IPR, Immediate phase response; LPR, late phase response; vLPR, very late phase response.
C, Control; P, predonisolone-21 acetate: 10 mg/kg, were given i.p. 2 h before and 4 to 6 days after the dinitrofluorobenzene (DNFB) challenge; cultivated: cultivated 4-year-old adventitious roots water extracts (GL-content: 5.63 ± 0.03%) ; Dongbei: Dongbei-Gancao extracts (GL-content: 9.71 ± 0.06%).
Samples were given (p.o) 2 h before and 2 to 6 days after the DNFB challenge.
*, $p<0.05$ vs control. (by Mann-Whitney's U-test).
【Modified from ref. 3)】

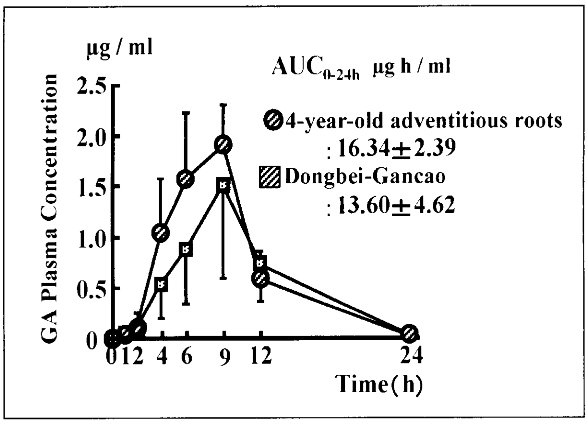

Fig. 26. GA plasma concentration-time profile after oral administration of water extracts of 4-year-old adventitious roots and Dongbei-Gancao
Each point and bar represent the mean ± SD (n= 5).
Each extract (⊘: 4-year-old adventitious roots; ▣ : Dongbei-Gancao) containing 45 mg/kg of GL was administered orally to rats.
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 plasma levels measured using the same HPLC conditions (Ref. 30a).
【Modified from ref. 3)】

evaluated by evaluating changes in GA in the plasma.[30)]

Water extracts of 4-year-old adventitious roots and medicinal licorice (Dongbei-Gancao) were orally administered to rats, and GA plasma concentration was serially measured until 24 hours after administration. The area under the mean concentration vs. time curve from zero to 24 hours ($AUC_{0\text{-}24h}$), maximum plasma concentration (C_{max}), and the time to reach C_{max} (t_{max}) of GA were calculated and compared between the two extracts. As shown in Fig. 26, after administration of water extracts of adventitious roots and medicinal licorice, similar GA plasma concentration-time curves were obtained. The C_{max} (1.90 ± 0.40 μg/ml) and $AUC_{0\text{-}24h}$ (16.34 ± 2.39 μg h/ml) of GA in 4-year-old roots did not significantly differ from those of medicinal licorice (C_{max}, 1.51 ± 0.91 μg/ml; $AUC_{0\text{-}24h}$, 13.60 ± 4.62 μg h/ml). These results suggested that 4-year-old *G. uralensis* roots are bio-equivalent to medicinal licorice used in Japan at present.

Thus, 4-year-old adventitious roots of *G. uralensis* cultivated in the eastern region of Neimenggu conformed to the JP XIV standard and were pharmacologically and biopharmaceutically comparable to medicinal licorice.[31)] These cultivated roots can be a resource that compensates for the insufficiency of medicinal licorice due to restrictions of the collection of wild *Glycyrrhiza* species.

5. General discussion and conclusion

Pharmacognosy deals with knowledge of crude drugs. The knowledge of crude drugs includes physical knowledge such as plants as resources and the composition and contents of components and the knowledge of the pharmacological effects and pharmaceutical characteristics of crude drugs as "medicaments". The two types of pharmacognostical knowledge are inseparable and should be clarified for the development and evaluation of cultivated resources as new medicinal resources. We performed pharmacognostical studies of *G. uralensis* cultivated in Neimenggu and obtained the following results.

1. To compensate for resource insufficiency due to restrictions of the collection of wild *Glycyrrhiza* plants in China, we reported the results of our survey of the status of *G. uralensis* cultivation as a measure against this insufficiency. The GL content of 3-year-old cultivated roots collected by our survey in cultivation districts did not conform to the JP XIV standard (≥2.5%).

2. Based on the results of this survey, we performed experiments of *G. uralensis* cultivation in the eastern region of Neimenggu (one of the sources of Dongbei-Gancao). In spring in the next year of seeding, taproots were transplanted, and adventitious roots of *G. uralensis* in autumn in the 4th year after seeding fulfilled the several standards including GL content of the JP XIV.

3. These cultivated roots were comparable to medicinal licorice used at present in terms of composition evaluated by multivariate analysis of HPLC profiles, anti-allergic effects, and the biological utilization rate of GL.

This study may be the first step to the medicinal use of cultivated roots to compensate for the insufficiency of licorice resources expected in the future. At present, licorice is still stably supplied, and excessive apprehension about resource insufficiency is not appropriate.

This study is also a study on the phyto-remediation of the global environment using cultivated plants. If cultivated roots are put to practical use, the collection amount of wild *Glycyrrhiza* plants can be reduced, which may contribute to the prevention of desertification in China.

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