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Pharmacognostical Studies on the *Clematis* Plants and Related Crude Drugs (III)¹⁾ On "Wei-ling-xian" (威霊仙) from Korean Peninsula²⁾

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An adequate amount of "Weilingxian" (威霊仙) is produced in Korean peninsula, and it is exported to Hong-kong, Taiwan and Japan. To clarify the botanical origin of "Weilingxian" from this area, comparative anatomical studies of the roots of the crude drug and three wild *Clematis* species of Ranunculaceae from Korean peninsula, *i.e. C. terniflora* DC., *C. terniflora* DC. var. *koreana* (NAKAI) TAMURA and *C. brachyura* MAXIM., were made. As the results, it was made clear that the underground part of all the three species were available as "威霊仙" from Korean peninsula, on Korean, Hong-kong, Taiwan and Japanese markets.

Keywords——Weilingxian; Wiryongseon; Clematis terniflora; origin; Clematis terniflora var. koreana; Clematis brachyura; Ranunculaceae; anatomical stury

As described in the previous paper,¹⁾ most of "Ireisen" (威震仙) consumed in Japan are produced in Korea. Moreover, "Weilingxian" (威震仙)³⁾ from Korean peninsula is also available on Hong-kong and Taiwan markets.⁴⁾ Thus, an adequate amount of "Weilingxian" is produced from Korean peninsula. Though "Wiryongseon" (威震仙)³⁾ is regarded as the roots of *Clematis terniflora* DC. (-C. mandshurica RUPR.) and *C. brachyura* MAXIM. of Ranunculaceae,⁵⁾ there has been no confirmation on it. Through our morphological study on the roots of *Clematis* plants having fibrous roots from Korea, it was made clear that they were so similar that it was impossible to know the botanical origin of "Wiryongseon" macroscopically.

To clarify the botanical origin of "Wiryongseon," comparative anatomical study was made on the roots of wild *Clematis* common in Korea, *i.e. C. terniflora*, *C. terniflora* var. *koreana* and *C. brachyura*. At the same time, *Clematis* roots that had been mixed with the crude drug "Eong geong kwi" used in Korean folk medicine, also investigated. Moreover, the botanical origin of "Wiryongseon" from North Korea could be confirmed.

Materials⁶⁾

Clematis terniflora DC.: Yong-cheon (永川), 75088-75099, Oct, 13, 1975; Lin-tsu-tou (林子 頭), Liaoning, China, J. Higashi, Aug., 2, 1943.

Clematis terniflora DC. var. koreana (NAKAI) TAMURA: Mt. Sok-li (俗離山), Chung-chong-pukdo, 75115, Oct., 19, 1975; Kwang-neung (光陵), Kyong-gi-do, 75111–75113, Oct., 18, 1975; Arboretum of College of Agriculture, Seoul National University, An-yang (安養), Kyong-gi-do, 78025–78026, July, 23, 1978.

Clematis brachyura MAXIM.: Yong-cheon, 75087, Oct., 14, 1975; Arboretum' of College of Agriculture, Seoul National University, An-yang, 78021–78023, July, 23, 1978.

"Wiryongseon" from Korea (South Korea) was obtained from the following places: Pyong-wha Chinese clinic (平和漢医院), Seoul, Nov., 1976; Jae-il Chinese drug store (第一漢薬房), Dae-gue, Apr., 1971, Sept., 1973; Chen-hsing Chinese drug store (振興薬行), Chia-i, Taiwan, Jan., 1977.

"Wiryongseon" from North Korea was obtained from Wing-tai-hong (永大行), Hong-kong, Aug., 1977.

"Eong geong kwi" was obtained from the open stall at Yong-cheon, Kyong-san-puk-do, Oct., 1975.

Experimental

Clematis terniflora DC. (C. mandshurica RUPR.)

Botanical characteristics: This species is placed in sect. *Flammula*, subsect. *Rectae*, ser. *Rectae* of genus *Clematis* of Ranunculaceae.⁷⁾ This species is variable in appearances, and possesses some botanical varieties. The herb never grows so big as *C. terniflora* var. *robusta* growing wild in Japan. The stem seems to be annual, measuring 4–6 mm in diameter at the basal portion, and never grows into woody hard one. The vine is 1–3 m in height, the short vine elects inclinally and the long one creeps on the ground or covers the bush. The achene is well compressed, and is ovate to fusiform in outline.

Macroscopical characteristics of the root (Fig. 1-A): 30-100 or more fibrous roots arise from a woody, massive or gnarled cylindrical rhizome measuring 5-15 mm in diameter and 2-19 cm in length. The roots are 2-3 mm in fresh and 1-2 mm in dry in diameter. The surface of the fresh root shows brown to dark brown, and changes into more blackish color when dried. The fresh root is flexible and the dried one liable to break. The broken surface shows yellowish white or light brown. The root has no special odor and taste.

Microscopical characteristics of the root (Fig. 1, B–L): At d.p. \sim 50 mm (at a distance of 50 mm from the proximal end), the roots measure 1,400–2,500 μ m, the steles 220–1,400 μ m in dimeter. The percentage of the stele in the root by diameter ranges 14.7–56.0% (mean: 24.0). The fusiform ratio⁸⁰ ranges 0.92–1.51. The outermost layer is epidermis with round or tangential elliptical epidermal cells measuring 40–60 μ m in diameter, the outer cell wall measures 5–10 μ m in thickness (K). Exodermis beneath the epidermis is one layer of suberized cells measuring 40–80 μ m in diameter (K). The cortex consists of 10–20 layers of parenchyma cells with diameters of 70–120 μ m and lengths of 200–390 μ m. The cortical parenchyma cell is round in young root and tangential oblong in old ones in outline. A distinctive endodermis arranging 38–80 cells in a ring is recognized (L). The average number of the endodermal cells in a ring of the roots from each stock is in the range of 55–68, and it is normally fewer than 60. The phloem fiber is scarce, and if any below 10 in number. The xylem is common in the shapes of lenticular to dumbell-like,⁹⁹ at d.p. 50 mm (B-2, C-2, E). The vessels measure 35–90 μ m with average of 53.2 μ m and 50–90 μ m with average of 72.8 μ m, respectively. In the steles less than 800 μ m and more than 800 μ m in diameter, the vessels measure 35–85 μ m (average: 60.9) and 75–90 μ m (84.2), respectively. The tracheid is about 15–25 μ m in diameter. The simple starch grain is up to 12 μ m, 2–8 complex up to 16 μ m in diameter.

Clematis terniflora DC. var. koreana (NAKAI) TAMURA (... C. papuligera OHWI)

Botanical characteristics: The plant is larger than C. terniflora, the stem near rhizome is 5–7 mm in diameter, and the vine grows more than 5 m in height. As compared with C. terniflora DC. var. robusta (CARR.) TAMURA growing wild in Japan, var. koreana has a smaller herb. The vine of this species never grows into large woody lignified one. Papillae on the veins projecting on the lower surface of the leaflet are recognized.

Macroscopical characteristics of the root (Fig. 2-A): The roots are quite similar to those of C. terniflora except that the rootstock is a little larger and the surface of the dried roots shows generally black or purple black.

Microscopical characteristics of the root (Fig. 2, B-K): The anatomical characteristics are fundamentally the same as those of *C. terniflora*. The transverse section of the root at d.p. = 50 mm is circular in outline, measuring 1,350–2,800 μ m, their normal sizes are more than 2,000 μ m in diameter. The steles measure 260–1,410 μ m in diameter, and the percentage of the stele in the root is in the range of 15.8–50.4% (mean: 29.0), normal ratio is more than 20%. The outermost layer is epidermis consisting of circular or tangential oblong cells, measuring 35–50 μ m in diameter, and having thickened outer cell walls with thickness of 6–12 μ m (J). The exodermal cell is circular or tangential oblong, measuring 40–60 μ m in diameter (J). The cortical parenchyma cells measure 70–120 μ m in diameter at the middle of cortex. The endodermat ting consists of 54–98 cells, and 70–77 in the average from each stock, at d.p. = 50 mm. The phloem fiber rarely reveals, and below 5 in number if any. Well developed xylem distinguish this species from other, xylem often reveals symmetric triarch at d.p. = 50 mm (E). The vessels measure 50–110 μ m (mean: 71.0) and 55–110 μ m (84.4) in diameter respectively at d.p. = 50 mm. In the steles less than 800 μ m, and more than 800 μ m, the vessels 50–110 μ m in diameter, respectively. Simple starch grains are up to 10 μ m, and complex grains up to 12 μ m in diameter in the middle of cortex.

Clematis brachyura MAXIM.

Botanical characteristics: The species was placed in sect. Pterocarpa of the genus. Only one species in this section grows in oriental Asia. The herb is botanically similar to that of *C. terniflora* except blooming fewer flowers in an inflorescence and bearing larger achene measuring nearly 10 mm in height and nearly 7 mm in width. The vine is similar to that of *C. terniflora*.

Macroscopical characteristics of the root (Fig. 3-A): The woody rugged rhizome is massive or gnarled cylindrical with diameter of 5–15 mm and length of 3–10 cm. The large rhizome produces more than 100 fibrous roots measuring nearly 2 mm in a fresh root and 1–1.5 mm in dry one in diameter, 20–40 cm in length. The dry root can

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Fig. 1. Clematis terniflora DC.

A: A sketch of "Wiryongseon" derived from this species. B-H: Anatomical variations in the transverse sections of the roots. I: Changing pattern of the vascular bundle from near rhizome to distal. J: Isolated elements; 1, wood parenchyma cell; 2, endodermal cell; 3, 4, pericyclic sclerenchyma cells; 5, phloem fiber; 6, pitted vessel; 7, tracheid; 8, wood fiber. K, L: Detailed drawings of the outer part of the root (K) and a part of stele (L) (The parenthesized numeral indicates the distance from the proximal end of the root; mm).



Fig. 2. Clematis terniflora DC. var. koreana (NAKAI) TAMURA

A: A sketch of "Wiryongseon" derived from this species. B: Changing pattern of vascular bundle from proximal to distal of the root. C-F: Variation of the vascular bundle shape at d.p. -50 mm. G, H: Changes of xylem elements from near rhizome to distal. I: Isolated elements; 1, pitted vessel; 2, tracheid; 3, wood parenchyma cell; 4, 5, wood fibers. J, K: Detailed drawings of the outer parts of the roots (J) and a part of stele in standard type at d.p. = 50 mm.



Fig. 3. Clematis brachyura MAXIM.

A: Sketches of "Wiryongseon" derived from this species. B-D: Diagram illustrations showing the changing pattern of the root structure from proximal to distal on the transverse section. E-G: Diagram illustrations showing the changes of the xylem elements. H: Abnormal large root. I-M: Variations of the transverse sections at d.p. = 50 mm.



Fig. 4. Clematis brachyura MAXIM.

A, D: Detailed drawings of the outer part of the root (A) and a part of stele (D) at d.p.=50 mm. B: Primary phloem and its surroundings at d.p.=5 mm. C: Isolated elements; 1-4, sclereids in pericyclic and phloem areas; 5, pitted vessel; 6, wood parenchyma cell; 7, tracheid; 8, wood fiber.

be broken easily, the outer surface of the root shows dark brown to black, and the broken surface shows nearly white or light yellow. The root scarcely has smell and has no taste.

Microscopical characteristics of the root (Fig. 3, B–H; 4): Anatomical characteristics of the root are more or less similar to those of *C. terniflora*. The diameters of the roots are somewhat larger, 1,600–3,100 μ m in diameter at d.p. = 50 mm, normally 1,900–2,500 μ m. The fusiform ratio is in the range of 0.95–1.43. The steles measure 260–2,000 μ m in diameter, the percentage of the stele in the root ranges 15.0–67.9%. The outermost layer is epidermis with round or tangential oblong epidermal cells, measuring 35–50 μ m in diameter (4-A). The outer cell wall is 5–8 μ m in thickness. In the root, of which surface is blackish color, the epidermal cell sometimes decay and fall off. The exodermal cell, measuring 45–70 μ m in diameter, has a wholly suberized cell wall (4-A). The cortical parenchyma cell is 70–120 μ m in diameter, arranging in 12–20 layers at d.p. = 50 mm. The endodermal ring consists of 54–117 (mean: 75.1) cells, and the average of the number from the roots of each stock is in the range of 66–92, normally 70–85, at d.p. = 50 mm. In the pericyclic and phloem areas, no sclereid appears in general,

and if any it is fewer than 10 cells at d.p. 50 mm. Xylem is lenticular to oblong in general at d.p. 50 mm (Fig. 3, B-2, D-2, E-2, I, J). The vessels measure 45-90 μ m in diameter. In the roots measuring less than 2,000 μ m, 2,000 3,000 μ m, and more than 3,000 μ m in diameter, at d.p. 50 mm, the vessels measure 45-67 (51.2), 45-85 (55.8), and 85-90 μ m in diameter, respectively. In the steles measuring less than 800 μ m, and more than 800 μ m in diameter, the vessels 45 70 (53.8) and 60-90 (76.7) μ m in diameter, respectively. The tracheids are smaller than those of *C. terniflora* in diameter, measuring 10-17 μ m. Simple starch grains in the middle of cortex measure up to 12 μ m, complex grains up to 14 μ m in diameter.

"Wiryongseon"

Morphological characteristics: The crude drug consists of a rhizome and many fibrous roots. The stem has been cut off in general. When the stem remains, it is about 3 mm in diameter and shorter than 3 cm in length. The roots are fibrous and cylindrical, arising more than 100 roots from a large rhizome, 1–2 mm in diameter, 5–25 cm in length. The surface of the root shows black, purple black, or dark brown, and sometimes light brown. At times, one rhizome bears roots of a variety of color. The root easily broken and the broken surface shows yellowish white to light brown. The crude drug has a slightly odor and has no taste.

Microscopical characteristics of the root: In the crude drug obtained from market, three types are recognized by the root anatomy. Each of them was confirmable to the root of *C. terniflora* DC., *C. terniflora* DC. var. *koreana* (NAKAI) TAMURA, and *C. brachyura* MAXIM. The botanical origin of "Wiryongseon" from Korea on Korean market was *C. terniflora* and *C. brachyura*, and that on Taiwan market was *C. terniflora* var. *koreana*. "Wiryongseon" from North Korea on Hong-kong market was derived from *C. terniflora* var. *koreana* and *C. brachyura*. The *Clematis* roots mixed with "Eong geong kwi" were derived from *C. brachyura*.

Results and Discussion

1. The anatomical characteristics in the roots of the three *Clematis* species growing wild in Korea, *i.e. C. terniflora* DC., *C. terniflora* DC. var. *koreana* (NAKAI) TAMURA and *C. brachyura* MAXIM., are shown in TABLE I. Each of the three species was characterized anatomically as follows: *C. terniflora* by its larger epidermal cell and tracheid, *C. terniflora* var. *koreana* by its larger vessel, *C. brachyura* by

ni da seren da seren de seren en seren en seren en seren de seren de seren de seren de seren de seren de seren	· · · · · · · · · · · · · · · · · · ·	C. terniflora	C. terniflora var. koreana	C. brachyura
Diameter of the root (µm)		1,400-2,500	1,350-2,800	1,600-3,100
Diameter of the stele (µm)		220-1,400	260-1,410	260-2,000
Percentage of stele in root by diameter $\binom{n}{2}$		14.7-56.0	15.8-50.4	15.0-67.9
Fusiform ratio ^a)		0.92-1.51	0.82-1.32	0.95-1.43
Epidermal cell Diameter (µm) Thickness of outer cell wall (µm)		40-60	35-50	35-50
		5-10	6-12	5-8
Cortex Diameter of cell (µm)		70-120	70-120	70-120
Number of cell layers		10-20	12-18	· 12–20
Increase of cell layersb)		0-5	1-4	0-4
Number of endodermal cells	each rooi	38-80	54-98	54-117
	average from one stock	55-68	70-77	66-92
Phloem fiber		rare; below 10	rare; below 5	rare; below 10
		if any	if any	if any
Diameter of vessel (µm)		35-90	50-110	45-90
		$(35-70, 75-90)^{\circ}$	(50-110, 95-110)	(45-67, 60-90)
Diameter of tracheid (µm)		15-25	15-20	10-17
Size of starch grain (µm)	Simple grain	up to 12	up to 10	up to 12
	Complex grain	up to 16	up to 12	up to 14

TABLE I. Anatomical Characteristics of the Roots of *Clematis* spp. from Korean Peninsula in the Transverse Sections at a Distance of 50 mm from the Proximal End

a) Diameter of the root at a distance of 50 mm from the proximal end, divided by that at 5 mm.

^{b)} Number of the cell layers at a distance of 50 mm from the proximal end, minus that at 5 mm.

c) The parenthesized numerals: the former shows the diameters of the vessels in the root measuring less than 2,000 μ m in diameter and the latter those in the roots whose steles are more than 800 μ m in diameter.

its smaller tracheid, respectively. As the results, it was made clear that "Wiryongseon" from Korea was derived mainly from the underground portions of *Clematis brachyura*, and rarely from those of *C. terniflora* and *C. terniflora* var. *koreana*.

2. "Wiryongseon" from North Korea was derived mainly from *C. terniflora* var. *koreana*, and rarely from *C. brachyura*.

3. Through our Korean market researches, it was made clear that most of "Wiryongseon" from Korea were a mixture of *C. brachyura* and another species. Through a survey on the wild *Clematis* plants, some *Clematis* species investigated in this study were growing in mixture in the same area. Especially these three species are quite similar to each other in the botanical characteristics. Herbal collectors cannot distinguish them when they dig up the roots. Though it was difficult to distinguish exactly the three species only by their appearances, the crude drug derived from the underground portion of *C. terniflora* var. *koreana* seemed to have a larger rhizome and more dark roots than those of the other two.

4. The roots of *C. brachyura* was mixed with the crude drug "Eong geong kwi" used in Korean folk medicine. "Eong geong kwi" is the crude drug derived from the roots of *Cirsium* plant of Compositae,¹²¹ the outline and odor of the roots are apparently different from those of *Clematis* plants. Therefore, it is hardly considered that the two kinds of roots have been mixed with together by mistake. If the two had been mixed by mistake, it must be happened when "Eong geong kwi" had been chosen from many kinds of roots collected by herbal collector, because "Wiryongseon" and "Eong geong kwi" are more or less similar to each other in their appearance when they are covered with soil or mud. It must be, however, more plausible to think that they had been mixed with together because of their common effects of diuretic and anodyne.

5. Though some other *Clematis* species having many fibrous roots are growing wild in Korea, those are *C. ianthina* KOEHNE, *C. patens* MORR. et DECNE., *C. fusca* TURCZ., it is considered that they might not come into the market as "Wiryongseon" because they are uncommon species in Korea. The anatomical characteristics of these species will be reported in the proceeding paper.

6. Clematis terniflora var. robusta growing wild in Japan, described in the previous paper,¹⁰ is a variety of C. terniflora. This variety is distinguished from C. terniflora botanically by its woody large stem. In the root anatomy, it is also distinguished easily by its larger roots and vessels, higher frequency of phloem fiber, etc.

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List of abbreviations: cx, cortex; dl, dark colored layer; en, endodermis; ep, epidermis; ex, exodermis; f, fiber; fb, fiber bundle; kl, cork layer; o, oil drop; p, parenchyma cell; php, primary phloem; pr, pericycle; s, sieb tube; sf, substitute fiber; sta, starch grain; t, tracheid; trc, transfusion cell; vp, pitted vessel; wp, wood fiber; wp, wood parenchyma cell; xp, primary xylem; xy, xylem.

References and Notes

- 1) Part II: M. Mikage, T. Namba, Shoyakugaku Zasshi, 37, 317 (1983).
- 2) A section of this work was presented at the 97th annual meeting of pharmaceutical Society of Japan, Tokyo, April, 1977.
- 3) In this paper, we use the term "Weilingxian" as a general name to marketed "威霊仙," and "Wiryongseon" to "威霊仙" from Korean peninsula.
- 4) As the result of our market researches.
- 5) M. Nakao, "Investigation of Chinese Drug in Tyosen (Korea)," Bulletin of The Shanghai Science Institute, Vol. II, Shanghai Science Institute, Shanghai, 1933, p. 87.
- 6) All the comparative plants were determined by Dr. M. Tamura, Faculty of Science, Kobe University.
- 7) M. Tamura, "Systema Clematidis Asiae Orientalis," Science Report, No. 4, Osaka University, Osaka, 1955, p. 33.
- 8) Diameter of root at d.p. 50 mm divided by that at 5 mm.
- 9) The previous paper¹⁰ is to be referred as to the development of the xylem.
- 10) T. Namba, M. Mikage, Shoyakugaku Zasshi, 37, 307 (1983).
- 11) The largest vessel in the section is measured.

12) J. Lee, "Korean Folk Medicine," Publishing center of Seoul National University, Seoul, 1966, p. 145; M. Kubo, Faculty of Pharmaceutical Science, Osaka University, Ph.D. Thesis, 1973, p. 125.

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