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A Comparative Study of Crude Drugs in Southeast Asia. X.[1] Crude Drugs derived from *Equisetum* Species

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This article reports histological study was made for crude drugs derived from *Equisetum* species. Six samples were examined and all were identified clearly: "S 378 mùzéi" (木賊), "KL 489 mùxì" (木夕), and "188 mùzéi in Formosa" (木賊, 台湾) were identified to be *E. hiemale* L. var. *affine* (ENGL.) A.A. EATON; "S 400 (S 611) jiégǔcǎo" (接骨草) and "KL 490 bógǔcǎo" (駁骨草) were *E. ramosissimum* DESF. *subsp. debile* (ROXB.) HAUKE; "30—40 cola de caballo" was *E. giganteum* L.

Keywords——histology of crude drugs; mùzéi; mùxì; jiēgǔcǎo; bógǔcǎo; cola de caballo; *Equisetum hiemale* L. var. *affine* A.A. EATON; *Equisetum ramosissimum* DESF. subsp. *debile* (ROXB.) HAUKE; *Equisetum giganteum* L.

It is known that in China the *Equisetum* plant (Equisetaceae) has been the source of two crude drugs. One is "wènjīng" (問荊), described in Běncǎoshíyí (本草拾遺) in 739 during the Tàng dynasty. The other is "mùzéi" (木賊), described in Jiāyòngběncǎo (嘉裕本草) in 1061 during the Sòng dynasty.

Both mùzéi and wénjīng have had various applications. Mùzéi, known as "jiégǔcǎo" (節股草), "jiējiēcǎo" (接接草) or "cuòcǎo" (挫草) in northeastern China, and as "tokusa" in Japan, comes from *E. hiemale* L., It has served as an astringent hemostatic, a diuretic, a diaphoretic, and as cure for eye-diseases.[3] Wènjīng is also called "jiēxùcǎo" (接續草) in China and in Japan "sugina", comes from *E. arvense* L.. It has been used as a hemostatic and as a diuretic.[3]

Both drugs, mùzéi and wènjīng, employ the aerial stem with and without membraneous leaves.

According to the reference, however, the source plant, its common name or commercial name and its usage differ somewhat. In Chángyòng-zhōngcáiyào-shǒucè (常用中草葯手冊),[4] the commonly used book on Chinese herbaceous drugs, *E. debile* ROXB. is quoted as the source for mùzéi. This reference states that mùzéi is used to cure intestinal catarrh and urinary calculus, and as a hyperpiesia *etc.*, It gives "jiéjiécǎo" (節節草), "bǐguǎncǎo" (毛管草) and "xiānruò-mùzéi" (紆弱木賊) as alternate names. In Shíyòng-zhōnyào-shǒucè (實用中葯手冊),[5] a book on the practical use of Chinese drugs, *E. hiemale* L. is quoted as the source for mùzéi and cuòcǎo, and *E. arvense* L. is for wènjīng, jiéjiécǎo and "bǐtóucài" (笔頭菜).

More differences appear in another reference, the Iconographia Cormophytorum Sinicorum, Tomus I (中国高等植物図鑒第一冊).[6] Three species of *Equisetum* are illustrated:

1) Part IX: A. Nitta, *South East Asian Studies*, 13, 641 (1976).
2) Location: *a) Shimoadachi-cho, Sakyo-ku, Yoshida, Kyoto; b) Senri Expo Park, Suita, Osaka; c)* Present address: *Kita Public Health Centre, Osaka.*
3) "Yàocáixué" (葯材学) ed. by Nánjīng-yàoyuéyuàn-yàocáixué-jiāoyánzu, Rénmín-wèishēn-chūbǎnchè, Peking, 1960, pp. 1050—1051.
4) "Chángyòng-zhōngcáiyào-shǒucè" (常用中草葯手冊), Shángwùyìn-shūguǎn, Hong Kong, 1970, pp. 694—695.
5) "Shíyòng-zhōngyào-shǒucè" (實用中葯手冊), Shángwùyìn-shūguǎn, Hong Kong, 1971, pp. 338—339, 422—424.
6) "Iconographia Cormophytorum Sinicorum, Tomus I," ed. by Zhōngguó-hèxuéyuàn-zhíwù-yánsuǒ, Kēxué-chūbǎnshè, 1972, pp. 116—117.

1) *E. hiemale* L. called mùzéi, cuòcǎo, jiéjiécǎo and "bǐtòucǎo" (笔頭草), is used as an astringent hemostatic, a diuretic, a diaphoretic and a cure for eye-diseases, and *E. debile* Roxb. is stated as a similar species with distribution in southern and southwestern region and along upper and middle parts of the Chángjiáng river; 2) *E. ramosissimum* Desf. called jiéjiécǎo, "tǔmáhuáng" (土麻黃), "cǎomáhuáng" (草麻黃) and "mùzéicǎo" (木賊草), is used as a diuretic and for eye-diseases, and *E. diffusum* Don is similar; 3) *E. arvense* L. called wènjīng, tǔmáhuáng, bǐtóucǎo and "mǎcǎo" (馬草), is used as an aid for cough, and as a hemostatic and diuretic. This plant is poisonous for domestic animals. Two similar species are *E. pratense* Ehrh., known as "cǎowènjīng" (草問荊), in which the sporule stem is green and branched, and *E. palustre* L., known as "quǎnwènjīng" (犬問荊), in which the nutritive stem grows simultaneously with the green sporule stem.

Thus, the inconstancy in common name has led to the confusion of the various species of *Equisetum*.

There are many reports in the fields of systematics dealing with *Equisetum* species. Eames,[7] Ogura,[8] *etc.* made discussion on the phylogeny of *Equisetum*, not on the species problem.

In 1963, Hauke published a revision of subgenus *Hippochaete*.[9] He tried to elucidate the natural hybrids chiefly based on histology. Eleven years later, in 1974, Namba, *et al.* reported in detail on commercial samples of Formosan and Japanese folk medicine.[10] Curiously, they did not refer Hauke's paper. The Namba's paper does not clearly identify the origin of some of these commercial drugs.

In the present paper it is intended to supplement Namba's work showing an appropriate method of identifying the origin of crude drugs.

Namba, *et al.* neglected Reimer's opinion (1854),[11] which was recognized two sections of *Equisetum*, section *Euequisetum* and section *Hippochaete*.

The differences between these two sections are found in the stomata and distribution area on the earth. The stomata of section *Hippochaete* are sunken inward from the surface of the stem; *Hippochaete* is generally evergreen and is distributed in tropical or subtropical zone. Section *Euequisetum* is distributed in the temperate zone and green only in the warmer season; The stomata are on the same surface of the stem. *Euequisetum* is considered as a newer section, and more species are belonged here.

Based primarily on such a difference between *Hippochaete* and *Euequisetum*, additional research is possible to finalize the determination of the species used in commercial drugs.

In this investigation 6 commercial samples, 5 from Southeast Asia as well as 1 from South America, were examined histologically. Four of the six samples are Chinese crude drugs collected in Southeast Asia in 1971 by Niita and Yoshida. Another samples, labelled "mùzéi in Formosa" (木賊, 台湾) is kept at the laboratory of pharmacognosy of the Faculty of Pharmaceutical Sciences, Kyoto University. The last sample was collected at Juliaca, southern part of Peru, 1966 by Yoshida. Its Spanish name is "cola de caballo" which literally means horsetail in English. Cola de caballo is used as a diuretic for renal disease, according to Quechuan (native Peruvians) which Yoshida personally interviewed.

Hippochaete which has 14 species including 7 hybrids are known in fewer species than *Euequisetum*.[12] It is ,therefore, rather easy to name the species of the samples in quesition based on morphological characters and distribution.

7) A.J. Eames and L.H. MacDaniels, "An Introduction to Plant Anatomy," 2nd ed., McGrow-Hill, New York, 1947, p. 135.
8) Y. Ogura, "Handbuch der Pflanzenanotomie," ed. by K.K. Linsbauer, G. Tischler, and A. Pascher, Band 7, Verlag von Gebrüder Borntraeger, Berlin, 1938, pp. 243—257.
9) R. Hauke, *Beihefte zur Nova Hedwigia*, **8**, 1 (1963).
10) T. Namba, T. Tani, and M. Kubo, *The Journal of Japanese Botany*, **49**, 138 (1974).
11) "A. Englers', Syllabus der Pflanzenfamilien I Band," Gebrüder Borntraeger, Berlin, 1954, pp. 285—286.
12) C. Reed, "Index to Equisetophyta, Part II, Extants, Index Equisetorum," Reed Herbarium, Baltimore, 1971, pp. 1—128.

"S 378 mùzéi" (木賊), "KL 489 mùxì" (木夕) and "188 mùzèi in Formosa" (木賊, 台湾) are possibly determined as *Equisetum hiemale* L. var. *affine* (Engl.) A. A. Eaton; "S 400 (S 611) jiēgǔcǎo" (接骨草) and "KL 490 bógǔcǎo" (駁骨草) may be referred either to *E. ramosissimum* Desf. subsp. *ramosissimum* or subsp. *debile* (Roxb.) Hauke; and "30—40 cola de caballo" can be considered either as *E. giganteum* L. or as *E. myriochaetum* Schlecht et Cham.

Experimental

Commercial Samples——1: S 378 mùzéi, collected at Ng Teck Sian pharmacy, Singapore, May 20., 1971, Aya Nitta, Shuji Yoshida.

2: KL 489 mùxi, collected at Men Tong pharmacy, Kuala Lumpur, Malaysia, June 12., 1971, Aya Nitta, Shuji Yoshida.

3: 188 mùzéi in Formosa, kept at the laboratory of pharmacognosy of the Faculty of Pharmaceutical Sciences, Kyoto University.

4: S 400 (S 611) jiégǔcǎo, same pharmacy, Singapore.

5: KL 490 bógǔcǎo, same pharmacy, Kuala Lumpur, Malaysia.

6: 30—40 cola de caballo, collected at Juliaca, Peru, Aug. 10., 1966, Shuji Yoshida.

Morphology——1, 2 and 3: These stems were hollow of green or yellow color. The standard for cutting each group of these samples differed slightly by case. Samples of 1 were thick-walled and the nodes were removed. Samples of 2 were cut at the nodes and some broken sheath remained, but the stem was not so thick-walled. Samples of 3 were cut irregularly, some without node and some up to 3 nodes, and the stem was thin-walled. Characteristics of these group of samples were an unbranched stem with an internodal length of 7 to 8 cm in samples of 2, and 5 to 6 cm in samples of 3. The nodal sheath was appressed and close against the stem, dark brown in color with a length the same or less than the diameter. Stomata formed in a single line on each side of a groove.

The surface of the stem was covered with silica, and scattered with silica grains.

4 and 5: Samples were cut at random into 5 to 7 cm pieces. The stems were hollow of green color with 1 to 4 whorls branching irregularly at each node. The diamter of the main stem was 3 to 4 mm, and branches were 1 to 2 mm. The length of the internode was 4 cm on the main stem, and 2 to 3 cm on the branch. The leaves had been transformed into a nodal sheath of funnel-form, elongated about twice the diameter. Sometimes a black belt was found, although not constant in width or position. Sheath teeth were membraneous, of grey in color, with apiculate tips, which had been broken off irregularly.

Stomata formed a single line each side of a groove. The ridges usually had small cross-bands of silica, the grooves had flat-topped rosettes in close rows.

6: Samples were cut at random. The main stem was big, 1 to 2 cm in diameter, with many regular whorls per node. The internodal length of the main stem was 8 cm, of the branch, 4 cm. The distance from the node of main stem to the first node of the branch was 1.5 cm. Nodal sheath of the main stem was cylindrical, the outer surface being grey and inside purple-brown with a length of 2 cm. The sheath teeth were black or dark brown, 1 cm in length. In some cases the branched stem rebranched to duplicate the original. The nodal sheath of branched stem was 3 to 4 mm in length. Stomata were arranged in 2 to 4(5) lines on each side of a groove. The surface of stem was similar to samples of 1, 2 and 3.

Histology——The 6 samples were observed in microscopy, revealing 3 types with the same grouping when examined histology and morphology.

1, 2 and 3: When seen in cross section of the stem the stomata were sunken from epidermis. The ridges were low and of gradual slope, having two rows of silica tuberculate with a median furrow separating them. The vallecular canal was tangential oval, 570 to 650 $\mu \times 510$ to 580 μ. Endodermis was double and common. Hypodermis consisted of collenchyma extending to the vascular bundle, but vallecular collenchyma were small, only a few cell layers.

The ratio of the hypodermis of the vallecular to that of the carinal was about 1: 10. (Fig. 1, Fig. 2, A).

4 and 5: In cross section the ridges were conspicuous and thrust out, the grooves had flat-topped rosettes, sunken stomata. The vallecular canals were tangential oval, 510 to 580 $\mu \times 270$ to 330 μ. The hypodermis was well developed extending both to the vallecular canals and vascular bundles. The ratio of the vallecular to carinal was 1: 3 to 1: 2. (Fig. 2, C).

6: In cross section the ridges and the grooves both had flat-topped rossettes, and the stomata were sunken. The vallecular canal was radial oval, 300 to 350 $\mu \times 750$ μ. The endodermis surrounded each vascular bundle. The hypodermis was the most developed among the 6 examined crude drugs and reached the endodermis and vallecular canal. The ratio of the vallecular to carinal being about 1: 3. (Fig. 2, D).

Specimens——1: *Equisetum hiemale* L. var. *affine* (Engl.) A.A. Eaton, in Japanese "tokusa," Mar. 1., 1974, cultivated in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Kyoto University.

2: *E. ramosissimum* Desf. subsp. *debile* (Roxb.) Hauke (=*E. debile* Roxb.) Dec. 15., 1965, near Mae Klang waterfall, inferior of Chom Thong, *ca.* 350 m alt., Chiangmai, Payap, Thailand, Motoji Tagawa, Kunio Iwatsuki and Nobuyuki Fukuoka No. 2263.

3: *E. ramosissimum* DESF. subsp. *ramosissimum* (=*E. ramosissimum* DESF.), May 25., 1942, Gyosanzan, Peking, China, Makoto Togashi, No. 516.

4: *E. ramosissimum* DESF. subsp. *ramosissimum* (=*E. ramosissimum* DESF. var. *japonicum* MILDE), in Japanese "inudokusa," June 14., 1972, along Muko river, Nishinomiya, Hyogo prefecture, Japan, Aya Nitta No. 12919.

5: *E. giganteum* L., July 10., 1947, Dept. Antioquia, 1560 m alt., in wet ditch near Medelin, Colombia, collector unknown.

6: *E. gigantheum* L. (=*E. martii* MILDE), Nov. 4., 1939, Guararapes-N.O.B.-, Figueila, Sañ Paulo, Brasil, Goro Hashimoto.

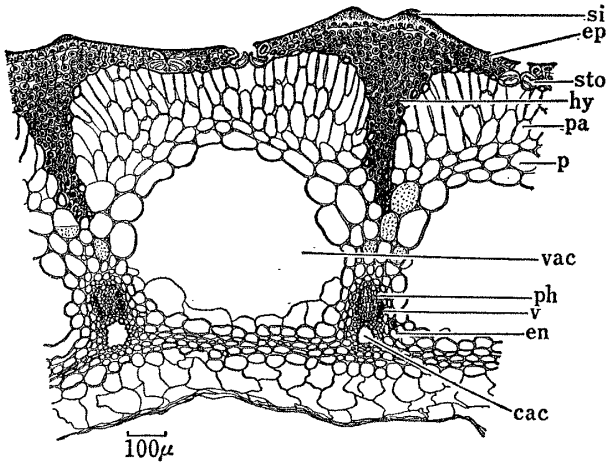

Fig. 1. Inner Structure of *Equisetum hiemale* L. var. *affine* (ENGL) A.A. EATON

7: *E. myriochaetum* SCHLECHT et CHAM. (labelled *E. giganteum* L.), Feb. 16., 1938, Wetlae river, alt. 900 m, on open river bank, Vera Cruz, sent from the Herbarium of E.B. Copeland.

Morphology and Histology——1: In all aspects the specimens were the same as commercial samples of 1, 2 and 3.

2: Almost all characteristics in morphology and histology were the same as commercial samples of 4 and 5.

3 and 4: These two specimens were the same in morphology and histology.

Stem was usually branched 2 to 3 per node. The nodal sheath was funnelform, its length being slightly more than twice its width. Stomata were in bands of 1 to 3 lines. In cross section carinal collenchyma usually extended to the endodermis but the vallecular collenchyma ranged nearly absent to quite extensive, according to age. The ratio of vallecular to carinal collenchyma was about 1: 2. The endodermis was double, with an inner endodermis and an outer endodermis surrounding all vascular bundles (Fig. 2, B).

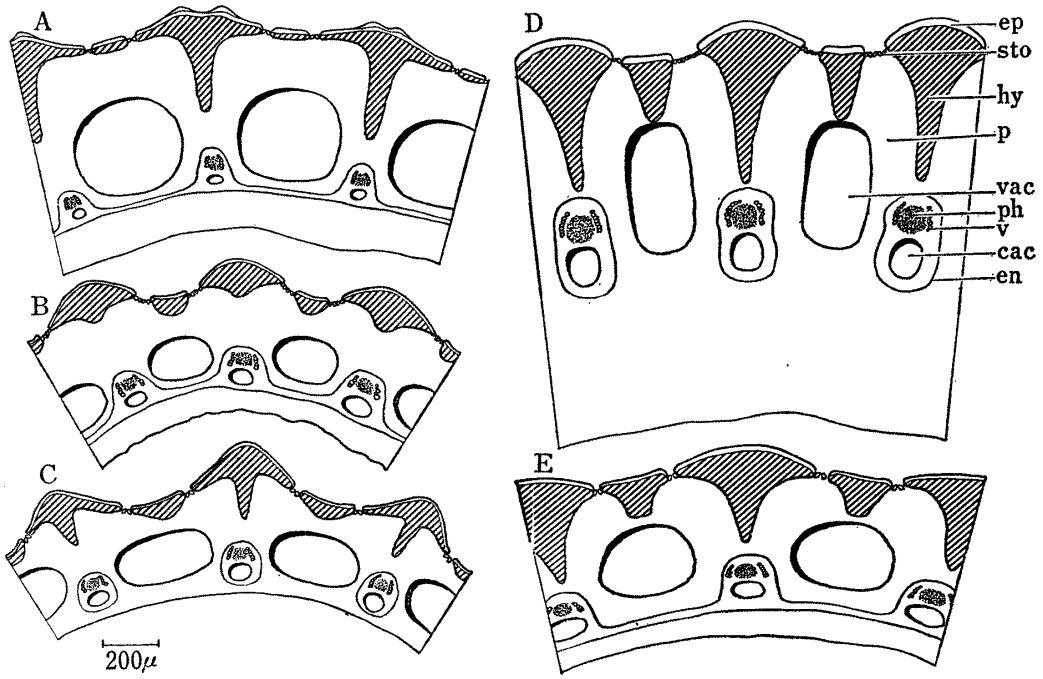

Fig. 2. Inner Structure of Commercial Samples and the Related Specimens of *Equisetum*

A: commercial samples 1, 2, 3 and *E. hiemale* L. var. *affine*
B: *E. ramoscssimum* DESF. subsp. *ramosissimum*
C: commercial samples 4, 5 and *E. ramosissimum* DESF. subsp. *debile*
D: commercial sample 6 and *E. giganteum* L.
E: *E. myriochaetum* SCHLECHT et CHAM.

5 and 6: These specimens were the same as commercial sample of 6.

7: Stomatal arrangement was a single line. In cross section the ridges were low and gradual slope. The vallecular canals were tangential oval, 420 μ×300 μ. The endodermis was double and common. The hypodermis extended both to the endodermis and to the vallecular canal. The ratio of vallecular to carinal collenchyma was 2: 3 (Fig. 2, E).

TABLE I. Histological Characteristics of Commercial Samples and the Related Specimens of *Equisetum*

Sample	Stomatal arrangement	Ratio of hypodermis	Endodermis	Specimen
S 378 mùzéi (木賊) K 489 mùxì (木夕)	1	1:10	double, common	*E. hiemale* L. var. *affine* (ENGL) A.A. EATON
188 mùzéi in Formosa (木賊, 台湾) S 400, S 611 jīegǔcǎo (接骨草) KL 490 bógǔcǎo (駁骨草)	1	1:3—1:2	individual	*E. ramosissimum* DESF. subsp. *debile* (ROXB) HAUKE
no sample	1—3	1:2	double, common	*E. ramosissimum* DESF. subsp. *ramosissimum*
30—40 cola de caballo	2—4(5)	1:3	individual	*E. giganteum* L.
no sample	1	2:3	double, common	*E. myriochaetum* SCHLECHT et CHAM

Result and Consideration

1) S 378 mùzéi (木賊), KL 489 mùxì (木夕) and 188 mùzéi in Formosa (木賊, 台湾) were identified as *Equisetum hiemale* L. var. *affine* (ENGL.) A. A. EATON.

As this plant is distributed in northern China and Japan, these crude drugs must be imported from China or Japan. According to this author's observation on cultivated *E. hiemale* L. var. *affine*, these 3 samples differ in thickness of stem at differed levels, *i.e.* upper part thin, lower part thick.

2) S 400 (S 611) jiēgǔcǎo (接骨草) and KL 480 bógǔcǎo (駁骨草) were identified as *E. ramosissimum* DESF. subsp. *debile* (ROXB.) HAUKE. As this plant is distributed from southern China to Southeast Asia, the crude drugs can be produced near the market, although the pharmacies reputedly imported these drugs from China. Additionally, in southern China, jiēgǔcǎo signifies *Genderusa vulgaris* NEES (Acanthaceae),[4] a drug different from the jiēgǔcǎo studied in this paper. The Acanthaceous plant of crude drug is also called "xiǎobógǔ" (小駁骨), "xiǎbógǔcǎo" (小駁骨草), "lǐlíqiáo" (裏禽樵) and "bǎijiécǎo" (百節草).

3) 30—40 Cola de caballo was identified as *E. giganteum* L. Cola de caballo is called "colai caballo" or "cawallo shupa" in Quechuan, and "tujchi wichchinca" in Aymaran. The distinct Aymaran name for this drug is important in that it indicates native use of the drug previous to the arrival of the Spanish and their influence. It is very interesting that *Equisetum* species were used in China and Peru independently but in similar ways.

4) In Iconographia Cormophytorum Sinicorum, Tomus I, 7 species of *Equisetum* distributed in China have been divided into 3 groups: 1) *E. hiemale* L. and the similar species *E. debile* ROXB.; 2) *E. ramosissimum* DESF., and the similar species *E. diffusum* DON; 3) *E. arvense* L. and the similar species *E. pratense* EHRH. and *E. palustre* L. The grouping seems to be made according to the various sizes of the plants, *E. hiemale* L. and *E. debile* ROXB. are much larger than *E. ramosissimum* DESF. and *E. diffusum* DON, which in turn, are larger than *E. arvense* L. and *E. pratense* EHRH., *E. palustre* L.

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List of abbreviations

si: silica tubercle, ep: epidermis, sto: stomata, hy: hypodermis, pa: palisade parenchyma, p: parenchyma, vac: vallecular canal, ph: phloem, v: vessel, en: endodermis, cac: carinal canal.