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Identification by Chemical Analysis of the Botanical Sources of Commercial Samples of Chinese Herbal Drugs

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ABSTRACT

The botanical sources of Chinese herbal drugs in a number of commercial samples could be inferred by comparing the contents of some characteristic constituents which were analyzed with high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE). The ratio of ephedrine/pseudoephedrine could be used as a marker to differentiate *Ephedra intermedia* from other species; the former is unique in having a value far less than unity. The total amounts of alkaloids in *Phellodendron wilsonii* and *P. amurense* var. *sachalinense* were more than 40 mg/g, which were two to three times more than in *P. amurense* and *P. chinense*. The contents of ginsenosides in *Panax notoginseng* and *P. quinquefolia* were generally higher than in *P. ginseng*, especially in the case of Rb_1.

Key words: **Chinese herbal drugs, chemical analysis, high-performance liquid chromatography, capillary electrophoresis.**

INTRODUCTION

Chinese herbal drugs are natural products which are subject to influence by such factors as the species, provenance, age of growth, harvest time and processing method. Among these factors, the species and provenance, which constitute the so-called "genuine drug" criteria, are conventionally held as the vital indices to good quality. Owing to the immense territory of China and the wide variety of complicated sources of herbs coming therefrom, there are homonymic herbal drugs coming from different botanical sources, and the therapeutic goals and uses of these homonymic herbs vary greatly. To date, the identification of Chinese herbal drugs in terms of their botanical sources still mainly relies on microscopic examination. In 1989 Kashiwada *et al.* succeeded in identifying the different species and provenances of rhubarb samples by comprehensive utilization of the assay values of 47 compounds including anthraquinones, anthrones, phenylbutanones, stilbenes, naphthalenes, flavan-3-ols, procyanidins, galloylglucoses and acetylglucoses[1]. By use of the HPLC or CE chromatographic patterns, it is possible to infer the botanical sources and to assess the quality of the commercial samples of Ephedrae Herba[2], Phellodendri Cortex[3], Coptidis Rhizoma[4], Ginseng Radix[5], Paeoniae Radix[6], Gardeniae Fructus and Evodiae Fructus[7].

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EPHEDRAE HERBA

Ephedrae Herba is a commonly used Chinese crude drug which possesses diaphoretic, antipyretic, antitussive and antiasthmatic effects [8]. It consists of the dried aerial parts of ephedraceous plants [8-12] and has been known to contain (-)-ephedrine, (+)-pseudoephedrine, (-)-methylephedrine, (+)-methylpseudoephedrine, (-)-norephedrine and (+)-norpseudoephedrine as its bioactive principles[12]. By means of capillary electrophoresis (CE)[13], the contents of these alkaloids have been determined in 22 commercial samples containing this herb and great differences were found among them[2].

The botanical origins of the 22 samples were categorized by their external appearances and histological anatomies and were identified as follows: eight of the samples were *Ephedra sinica* Stapf, six were *E. intermedia* Schrenk et Meyer, two were mixtures of *E. sinica* and *E. equisetina* Bunge, three were mixtures of *E. sinica* and *E. intermedia*, one was both *E. distachya* L. and *E. sinica*, another one was *E. distachya* and *E. intermedia* and still another one were *E. equisetina* and *E. intermedia*.

Investigation of the contents of the individual alkaloids revealed that the quantity of ephedrine was always the highest in all species except *E. intermedia*. The combined amounts of ephedrine and pseudoephedrine made up about 90% of the total alkaloids, whereas methylpseudoephedrine was the least in almost all the samples. The assay data plotted in Fig. 1 shows that samples of *E. sinica* and *E. intermedia* have clear differences in the ratios of contents of the constituents. From the characteristic pattern shown in Figure 1, Ephedrae

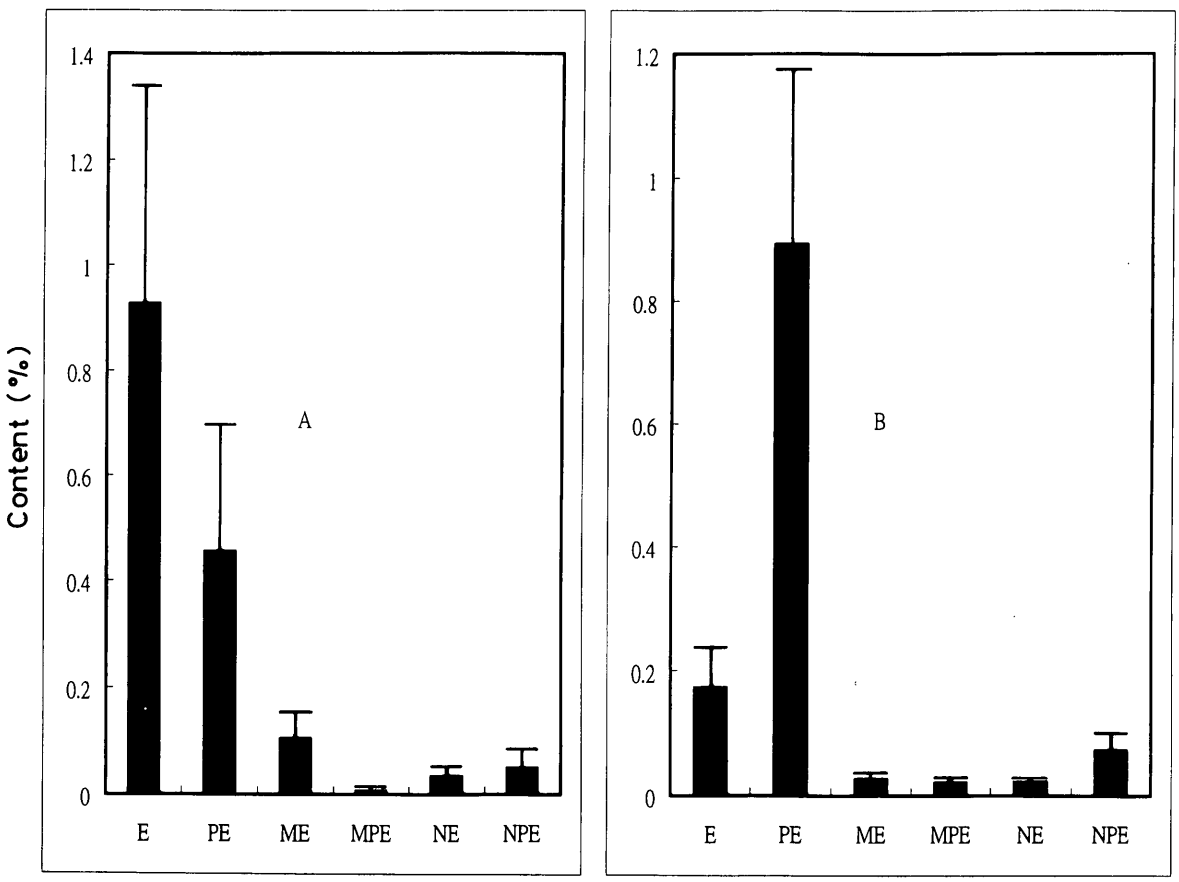

Figure 1. The contents of alkaloids in *Ephedra* spp.(A) *E. sinica* (n=8), (B) *E. intermedia* (n=6). E=ephedrine, PE=pseudoephedrine, ME=methylephedrine, MPE=methylpseudoephedrine, NE=norephedrine, NPE=norpseudoephedrine.

Herba was correctly indentified in several dozen commercial products in which it was present on its own or as one of the ingredients of an herbal formula. Commercial samples are often mixtures of two or more kinds of herbs of different sources; this is especially true for *E. equisetina* and *E. distachya*, which were never present singly (see Fig. 2). Definite patterns for the ratios of the three stereoisomeric pairs were obtained. The E/PE, ME/MPE and NE/NPE ratios in the samples of four species were: for *E. sinica*, E/PE > 1, ME/MPE > 10, NE/NPE > 0.4; for *E. intermedia*, E/PE < 0.3, ME/MPE ≈ 1, NE/NPE < 0.4; for *E. equisetina*, E/PE > 1, ME/MPE ≈ 10, NE/NPE ≈ 0.4; and for *E. distachya*, E/PE > 1, ME/MPE ≈ 5, NE/NPE < 0.4.

Although the patterns of the constituent ratios in the various samples were consistent, the absolute amounts of the constituents differed greatly from one to another. The total amount of the alkaloids ranged from 0.536% to 2.308%. Three of the samples had total amounts exceeding 2%, and six were between 1.5 - 2.0%. These samples were found to contain *E. sinica* or to be mixtures containing this species. Samples found to have total amounts below 1%, on the other hand, contained *E. intermedia*. Generally, in terms of total alkaloid content, *E. sinica* was superior (1.594 ± 0.467%) and *E. intermedia* was inferior (1.210 ± 0.372%) (Fig. 2). Assays showed that the total content of the six alkaloids in the internode was on average about four times that in the node and that the total amount in the thin-stemmed samples was about 1.5 times that in the coarse-stemmed ones. The constituents were also more concentrated in powdered form than in the fibrous from(by about 5:1). Therefore, with regard to quality, those samples that contain more thin stems with less nodes and fibers and that snap easily are superior.

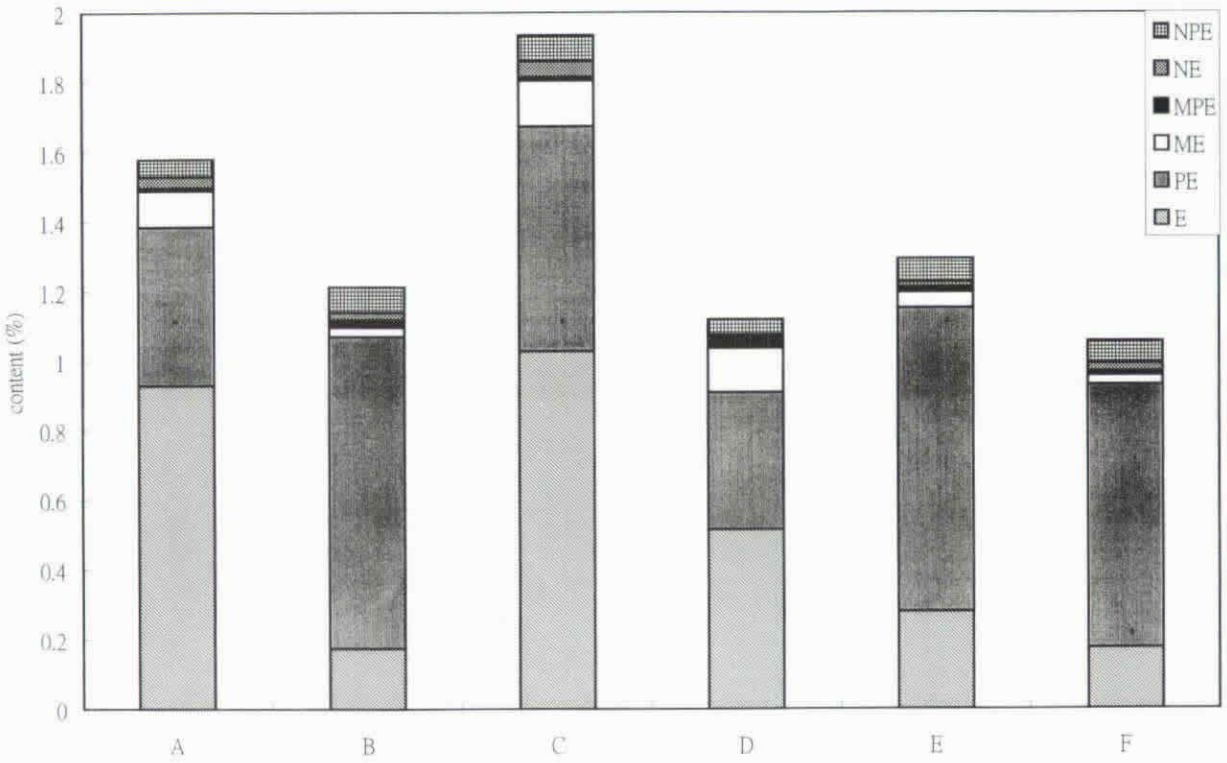

Fig.2 The total contents of alkaloids in various commercial Ephedrae Herba samples (A) *E. sinica* (n=8), (B) *E. intermedia* (n=6), (C) mixture of *E. sinica* and *E. equisetina* (n=2), (D) mixture of *E. distachya* and *E. equisetina* (n=1), (E) mixture of *E. sinica* and *E. intermedia* (n=3), (F) mixture of *E. intermedia* and *E. equisetina*(n=2).

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PHELLODENDRI CORTEX

Phellodendri Cortex, the dried trunk bark of a rutaceous plant, is a commonly used Chinese herbal drug able to clear heat, moisten aridity, purge fire, detoxify toxicosis and clear deficiency-associated fever[14]. It is known to contain berberine, palmatine, jatrorrhizine, phellodendrine, and magnoflorine as its bioactive principles[15-17]. With these compounds as the indexing standards, 31 samples collected from different herb shops in Taiwan and Japan and identified and categorized by pharmacognostic histological anatomy, were assayed by CE for their alkaloid contents[18]. The 31 commercial samples were found to have been derived from the following sources: fourteen samples were *Phellodendron chinense* Schneid, four samples were *P. amurense* Ruprecht, seven samples were *P. wilsonii* Hayata et Kanehira, and the other seven were derived from *P. amurense* Rupr. var. *sachalinense* Fr. Schm[3].

Investigation of the contents of the individual alkaloids revealed that berberine was the most abundant, except in some samples of *P. chinense* and *P. amurense* which contained more magnoflorine than berberine. Berberine comprised 85% of the total alkaloids in *P. wilsonii*, 75% in *P. amurense* var. *sachalinense*, 40% in *P. amurense*, and 37% in *P. chinense*. In capillary electropherograms, samples derived from the same botanical source had almost identical patterns (Fig. 3). The average values of the contents of total alkaloids, palmatine and phellodendrine and the ratios of total alkaloid/berberine and total alkaloid/magnoflorine in samples of the same species were also

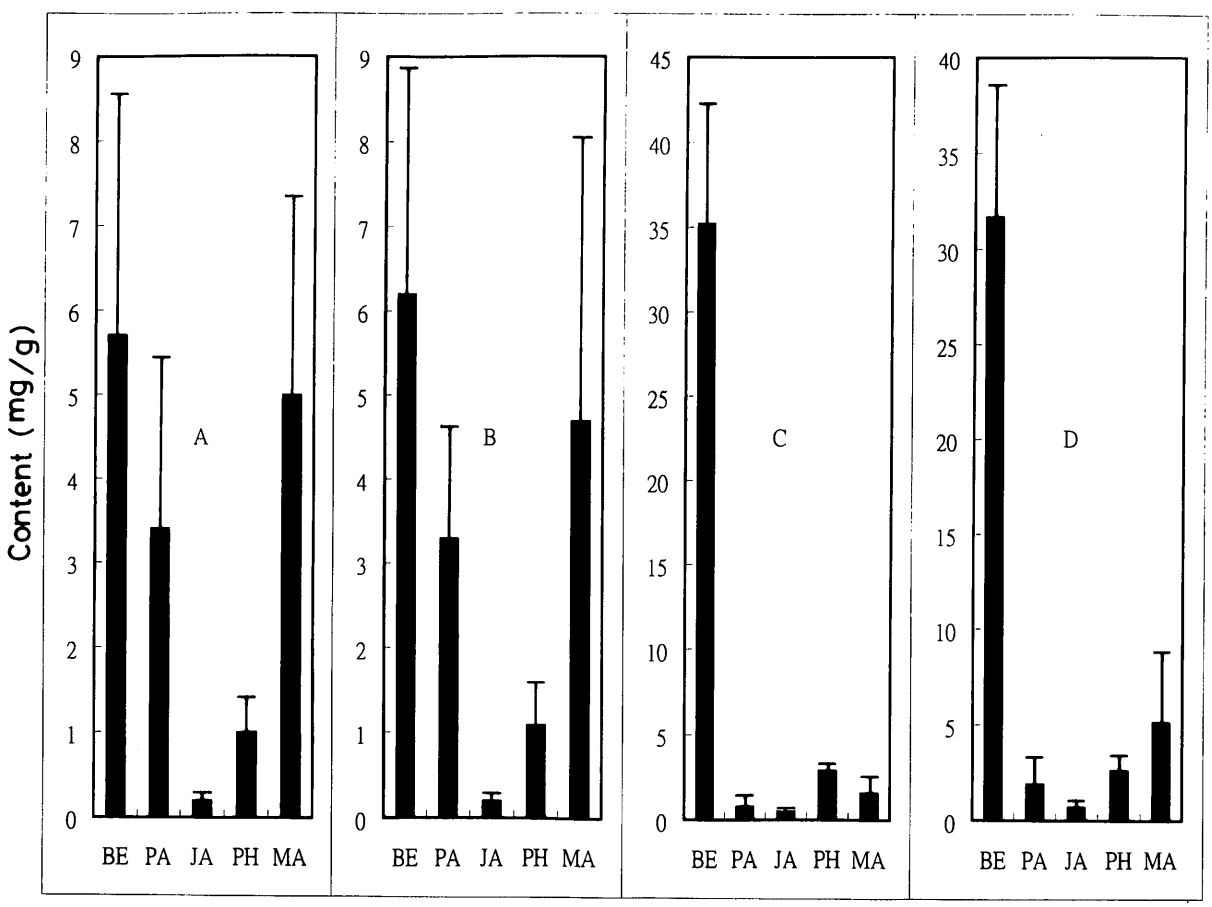


Figure 3. The contents of alkaloids in *phellodendron* spp. (A) *P. chinensis* (n=14),(B) *P. amurense* (n=4), (C) *P. wilsonii* (n=7), (D) *P. amurense* var. *sachalinense* (n=7). BE=berberine, PA=palmatine, JA=jatrorrhizine, PH=phellodendrine, MA=magnoflorine.

consistent. The most marked characteristics were the content of total alkaloids and the ratio of total alkaloids to magnoflorine. Total alkaloid content could be used to easily identify samples derived from *P. chinense*, *P. amurense* (≦15 mg/g) or *P. wilsonii*, *P. amurense* var. *sachalinense* (≧41 mg/g). The total alkaloid / magnoflorine ratio clearly distinguished between samples of *P. wilsonii* (≧25) and *P. amurense* var. *sachalinense* (≦8). *P. chinense* and *P. amurense* had almost identical electropherogrammatic patterns, which made their identification possible only by referring to the external appearance, fracture surface and phloem rays of the herbs. *P. chinense* had a deep-yellow fracture surface, a grey-brown outer cortex and curved phloem rays; *P. amurense* had a fresh yellow or yellow-green fracture surface, a grey-white cortical layer and straight phloem rays.

There were great differences in total alkaloid content among the samples, ranging from a low of 6.9 mg/g to a high of 53.3 mg/g. Three samples had contents above 50 mg/g and five samples were between 40 and 50 mg/g. All of these 8 samples contained either *P. wilsonii* or *P. amurense* var. *sachalinense*. Samples with total contents below 10 mg/g, on the other hand, were invariably those derived from *P. chinense* or *P. amurense*. As a result, samples of *P. wilsonii* and *P. amurense* var. *sachalinense* were found to be superior to those *P. chinense* and *P. amurense* (Fig. 4). Samples of the various *Phellodendron* species differed greatly in their external appearance. *P. wilsonii* and *P. amurense* var. *sachalinense* which were of better quality were more solid and dense in texture; whereas the two species produced in mainland China were comparatively more porous and lighter in weight. Generally, both within and across species, the more intensely yellow colored the fracture surface is, the better the herb's quality will be. *P. wilsonii* and *P. amurense* var. *sachalinense* had a more intense yellow color than *P. chinense* and *P. amurense*.

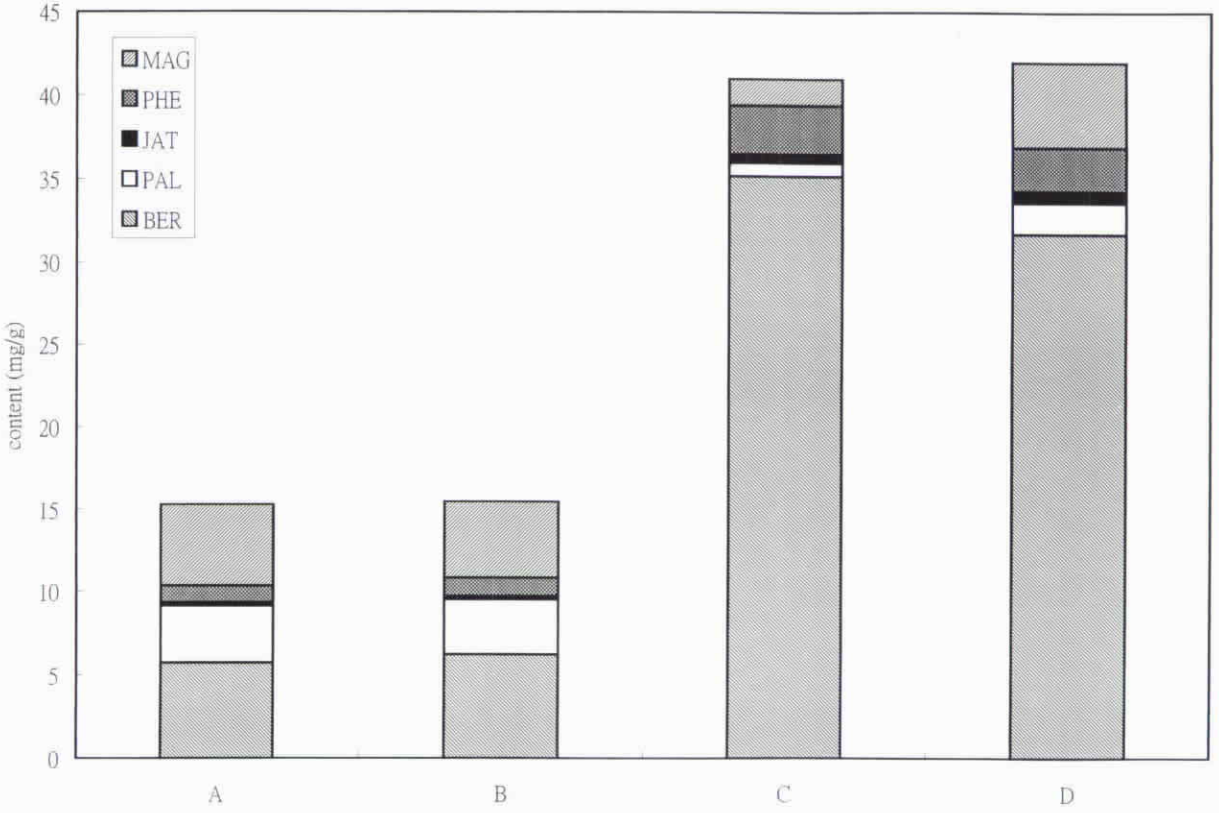
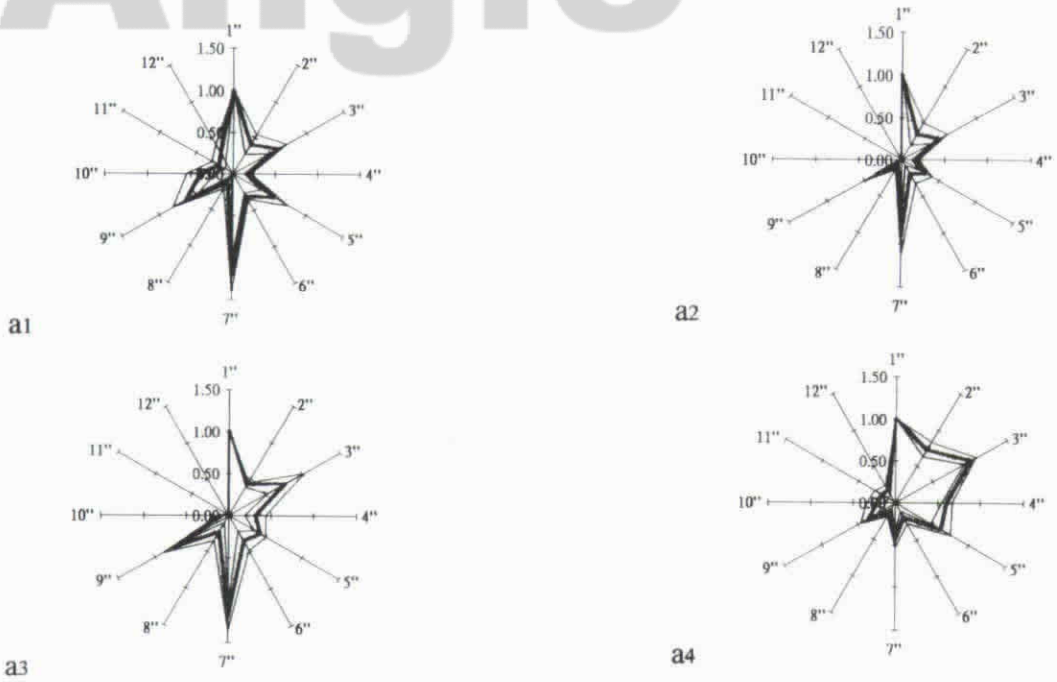


Figure 4. The total contents of alkaloids in various commercial phellodendri Cortex samples. (A) *P. chinensis* (n=14), (B) *P. amurense* (n=4), (C) *P. wilsonii* (n=7), (D) *P. amurense* var. *sachalinense* (n=6)

A: *P. ginseng* C. A. Meyer



B: *P. quinquefolia* Linn.

C: *P. notoginseng* Burkill

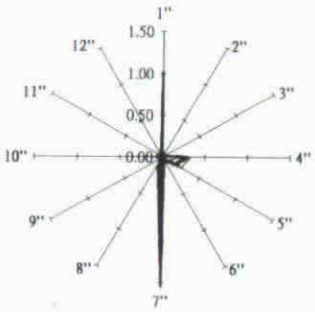


Figure 5. Patterns of the components ratios (ginsenoside/Rb_1) in *Panax* spp. (A) *P. ginseng* (a1: white-ginseng, n=10; a2:red-ginseng, n=5; a3:shihchu-ginseng, n=3; a4: ginseng root-hair, n=4). (B) *P. quinquefolia* (b1: wild American ginseng, n=4; b2: cultured American ginseng, n=6). (C) *P. notoginseng* (sanchi-ginseng, n=5). 1"=Rb_1/Rb_1, 2"=Rb_2/Rb_1, 3"=Rc/Rb_1, 4"=Rd/Rb_1, 5"=Re/Rb_1, 6"=Rf/Rb_1, 7"=Rg_1/Rb_1, 8"=Rg_2/Rb_1, 9"=R_0/Rb_1, 10"=mRb_1/Rb_1, 11"=mRb_2/Rb_1, 12"=mRc/Rb_1. ■,mean content; □, ± SD.

GINSENG RADIX

Ginseng Radix is one of the most commonly used Chinese herbal drugs and it possesses CNS-stimulating, cardiotonic and hypotensive effects [19]. The active constituents of this herb were found to be a complex mixture of saponins often referred to as ginsenosides, and more than 30 major ginsenosides have been documented so far. The pharmacological studies on ginseng have centered on the eight neutral saponins, ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, an acidic saponin, Ro, and three acidic malonates of the dammarane saponins, malonylginsenosides mRb_1, mRb_2, mRc, all of which are characteristic of *Panax* spp.[20-22]. Using these compounds as marker substances, 37 samples, which were col-

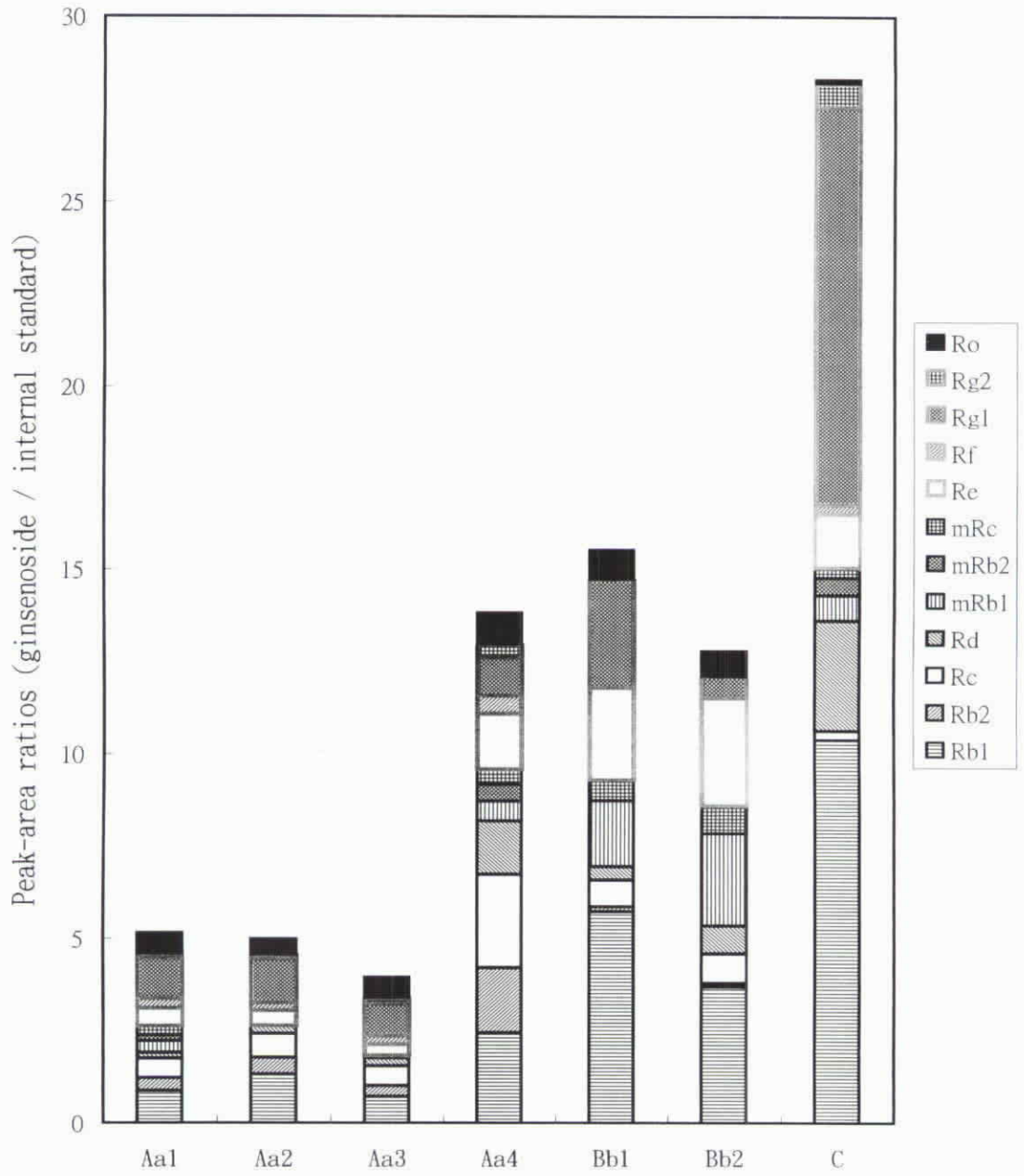


Figure 6. The total contents of ginsenosides in various Ginseng Radix samples (Aa1) white-ginseng (n=10), (Aa2) red-ginseng (n=5), (Aa3) Shihchu-ginseng (n=3), (Aa4) root-hair (n=4),(Bb1) wild American ginseng (n=4), (Bb2) cultured American ginseng (n=6), (C) Sanchi-ginseng (n=5).

lected from different herb shops in Taiwan, were analysed by high-performance liquid chromatography[23].The botanical sources of the 37 commercial samples were identified as follows: twenty-two were derived from *P. ginseng* C.A. Meyer (white-ginseng, red-ginseng, shichu-ginseng, and ginseng root-hair), ten were *P. quinquefolia* Linn. (wild American ginseng and cultured American ginseng) and five were from *P. notoginseng* Burkill (sanchi-ginseng)[5].

Samples derived from the same botanical source had very similar chromatogrammatic patterns. The characteristic ratios of each ginsenoside/Rb_1 for the different samples are shown in Fig. 5, from which it is easy to identify samples derived from either *P. ginseng*, *P. quinquefolia* or *P. notoginseng*. In terms of the content of total saponins, *P. notoginseng* (sanchi-ginseng, C) ranked the highest, followed by *P. quinquefolia* (American ginseng, B), root-hair (Aa4) of *P. ginseng*, red-(Aa2) and white-ginseng (Aa1) from *P. ginseng*, and finally the shihchu-ginseng (Aa3) from *P. ginseng*. The ratios were approximately 14:7:3:2 (C>(B and Aa4)>(Aa1 and Aa2)>Aa3) (Fig. 6). There are three major classes of ginsenosides: panaxadiol, panaxatriol and oleanolic acid. In terms of the total content of panaxadiol, sanchi-ginseng was the highest, followed in decreasing order by American ginseng and ginseng root-hair, and then the other three *P. ginseng* samples in the ratio 9:3:1 (C>(B and Aa4)>(Aa1 , Aa2 and Aa3)). Panaxatriol rankings were similar to thoses for panaxadiol and were in the ratio 5:3:1. By contrast, the decreasing rank order for oleanolic acid was American ginseng and ginseng root-hair, *P. ginseng* samples, and *sanchi*-ginseng in the ratio 4:2:1 ((B and Aa4)>(Aa1 , Aa2 and Aa3)>C). It is noteworthy that the ratio of Rg_1 to Rb_1 (Rg_1/Rb_1), two ginsenosides which have antigonistic pharmacological activities was far less than unity only in American ginseng and ginseng root-hair, which had values of 0.15 and 0.43, respectively.

Although the white-ginseng samples with bigger cross sections and a yellowish brown color were superior in quality, the red-ginseng which appeared as sticky granules after pulverization contained higher amounts of ginsenosides. Ginseng root-hair was the cheapest in price of all the *P. ginseng* products and yet it contained the highest amounts of the ginsenosides Rb_1 and Re. The Rb_1 content in ginseng root-hair was about 2 - 3 times higher than in other *P. ginseng* samples, but its Rg_1/Rb_1 ratio was the lowest (0.43). As for the *P. quinquefolia* and *P. notoginseng* samples, those derived from the wild species are of better quality than the cultured ones, while sanchi-ginseng that is big and black is of superior quality.

REFERENCES

1. Kashiwada, Y., Nonaka, G. I. and Nishioka, I. 1989. Studies on rhubarb (Rhei Rhizoma).XV. Simultaneous determination of phenolic constituents by high-performance liquid chromatography. Chem. Pharm. Bull. 37:999-1004..
2. Liu, Y. M., Sheu, S. J., Chiou, S. H., Chang, H. C. and Chen, Y. P. 1993.A comparative study on commercial samples of Ephedrae Herba. Planta Med. 59: 376-378.
3. Liu, Y. M., Sheu, S. J., Chiou, S. H., Chang, H. C. and Chen, Y. P. 1994. A comparative study on commercial samples of Phellodendri Cortex. Planta Med. 59: 557-561.
4. Liu, Y. M., Sheu, S. J., Chiou, S. H., Chang, S. H. and Chen, Y. P. 1994. Capillary electrophoretic analysis of alkaloids in commercial samples of Coptidis Rhizoma. Phytochem. Anal. 5: 256-260.
5. Chuang, W. C., Wu, S. K., Sheu, S. J., Chiou, S. H., Chang, H. C. and Chen, Y. P. 1995. A comparative study on commercial samples of Ginseng Radix. Planta Med. 61: 459-465.
6. Chuang, W. C., Lin, W. C., Sheu, S. J., Chiou, S. H., Chang, H. C. and Chen, Y. P. 1996. A comparative study on commercial samples of the roots of *Paeonia vitchii* and *P. lactiflora.* Planta Med. 62: 347-351.
7. Unpublished data.
8. China Crude Herbs Researching and Developing Center. 1974. "Modern Herbal-Chinese Material Medica". pp. 1051-1055.

Chi-yeh Book Store, Taipei, Taiwan.
9. Li, C. Y. 1959. "Pharmacognosy". pp. 57-61. Modern Medical and Pharmaceutical Press, Taipei, Taiwan.
10. Lai, J. H. 1976. "Colored Pharmacognosy". pp. 469-472. Chuan-i Press, Taichung, Taiwan.
11. Namba, T. 1980. "Chinese Herbal Drugs with Color Illustrations". Vol. 2. pp. 53-57. Boyo-kusha, Tokyo, Japan.
12. Hsu, H. Y., Chen, Y. P., Sheu, S. J., Hsu, C. H., Chen, C. C. and Chang, H. C. 1985. "Chinese Material Medica - A Concise Guide". pp. 31-32. Modern Drug Press, Taipei, Taiwan.
13. Liu, Y. M. and Sheu, S.J. 1992. Determination of ephedrine alkaloids by capillary electrophoresis. J. Chromatogr. 600: 370-372.
14. Hsu, H.Y., Chen, Y. P., Sheu, S. J., Hsu, C. H., Chen, C. C. and Chang, H. C. 1985. "Chinese Material Medica - A Concise Guide". pp. 117-118. Modern Drug Press, Taipei, Taiwan.
15. Tomita, M. and Nakano, T. 1957. Alkaloids of rutaceous plants. I. Alkaloids of *Phellodendron amurense* 1. Pharm. Bull. (Tokyo) 5: 10-12.
16. Tomita, M. and Kunitomo, J. 1958. Studies on the alkaloids of rutaceous plants. V. Alkaloids of *Phellodendron amurense* Pupr. var. *sachalinense* Fr. Schm. Yakugaku Zasshi. 78: 1444-1447.
17. Kunitomo, J. 1961. Studies on the alkaloids of rutaceous plants. XV. Alkaloids of *Phellodendron amurense* Pupr. var. *japonicum* Ohwi. (1). Yakugaku Zasshi. 81: 1370-1372.
18. Liu, Y. M. and Sheu, S. J. 1993. Determination of quaternary alkaloids from Phellodendri Cortex by capillary electrophoresis. J. Chromatogr. 634: 329-333.
19. Hsu, H. Y., Chen, Y. P., Sheu, S. J., Hsu, C. H., Chen, C. C. and Chang, H. C. 1986. "Chinese Material Medica - A Concise Guide". pp. 528-531, 596-597. Oriental Healing Arts Institute, Los Angeles, U. S. A.
20. Tanaka, O. and Kasai, R. 1984. "Progress in the Chemistry of Organic Natural Products". Vol. 46. p. 1. (Herz, W., Grisebach, H., Kirby, G. W. and Tamm, Ch. eds.), Springer-Verlag, New York, U. S. A.
21. Tanaka, O., Kasai, R. and Morita, T. 1986. Abstracts of Chinese medicines. 1: 130.
22. Kitagawa, I., Taniyama, T., Hayashi, T. and Yoshikawa, M. 1983. Chemical studies on crude drugs. I. On the constituents of Ginseng Radix rubra. Chem. Pharm. Bull. 31: 3353-3357.
23. Chuang, W. C. and Sheu, S. J. 1994. Determination of ginsenosides in ginseng crude extracts by high-performance liquid chromatography. J. Chromatogr. A. 685: 243-251.

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市售中藥材基原之化學鑑別

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摘　要

市售中藥材的基原可藉高效液相層析儀或毛細電泳分析比較其中某些特殊成分而推定。麻黃素與僞麻黃素的比值可作爲分辨不同麻黃品種的指標，該值在中麻黃遠小於1，但其他品種均大於1。生物鹼的總含量可用於判斷市售黃柏樣品的來源，台灣黃柏與日本黃柏均大於40 mg / g，但川黃柏與關黃柏則只有前述值的二分之一到三分之一。人參皂素，特別是Rb_1，含量在各種人參樣品中有很大差異，三七人參與美國人參遠高於高麗人參。同樣方法可適用於黃連、芍藥、梔子、吳茱萸等藥材。

關鍵詞：中藥材，化學分析，高效液相層析，毛細管電泳。