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Chemical Identification on the Origins of Phellodendri Cortex

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ABSTRACT

Twenty-eight samples of commercial articles of Phellodendri Cortex, originating from *Phellodendron amurense* $R_{UPRECHT}$, *P. chinense* S_{CHNEID}, *P. wilsonii* H_{AYATA} et $K_{ANEHIRA}$, and *P. chinense* S_{CHNEID}. var. *glabrius culum* S_{CHNEID}, were collected from different herbal markets in Taiwan and Mainland China. The five alkaloids berberine, palmatine, jatrorrhizine, phellodendrine, and magnoflorine were determined by capillary electrophoresis for their contents in the samples, thereby a chemical identification method was established for identifying the sources of the drug materials. As this study turned out, *P. chinense* var. *glabrius culum* was found to be the predominant variety prevailing in Taiwan markets owing to their considerably high alkaloid contents (18~38 mg/g), and was currently introduced into Taiwan. With the analytical results obtained in this study, characteristic fingerprint for the variety of *P. chinense* var. *glabrius culum* was made and the suitability of the previously established chemical identification methods in the identification of other species of the drug materials were further verified. In addition, a facile identification flowchart based on the various chemical identification methods for Phellodendri Cortex was devised.

Key words: Phellodendri Cortex, alkaloid content, capillary electrophoresis, species identification

INTRODUCTION

Phellodendri Cortex, the dried trunk bark of a Rutaceous plant, is commonly used in Chinese herbal medicine possessing the effects of clearing heat, moistening aridity, purging fire, detoxifying toxicosis, and clearing deficiency-fever[1]. It is known to contain berberine (BER), palmatine (PAL), jatrorrhizine (JAT), phellodendrine (PHE), and magnoflorine (MAG), Figure 1, according to its bioactive principles[2-5]. Since Phellodendri Cortex is very complicated with respect to its sources on the herbal drug market, a systematic, comparative study of alkaloid contents by using capillary electrophoresis on 31 samples was performed by Liu *et al*.[6]. In that study, 4 origins were identified (**I**, *Phellodendron wilsonii* H_{AYATA} et $K_{ANEHIRA}$; **II**, *P. amurense* $R_{UPRECHT}$ var. *sachalinense* $F_{R.}$ $S_{CHM.}$; **III**, *P. amurense* $R_{UPRECHT}$; **IV**, *P. chinense* S_{CHNEID}), and the total alkaloid contents (TA) of **I** and **II** was found to be superior (almost threefold) to that of **III** and **IV**. At that time, the latter two species were the most prevalent on the market. By keeping track of the herbal drug quality over the years, we found that the quality (in terms of berberine content) of Phellodendri Cortex samples on the market has been highly enhanced in recent years, and its origin was *P. chinense* S_{CHNEID} var. *glabrius culum* S_{CHNEID} (**V**) which was identified by the histological anatomy and microscopic observance[7]. In this study, 28 commercial samples were collected from herbal drug markets in Taiwan and the Mainland China to include as many origins as possible in order to establish the chemical fingerprint of **V**, and also to confirm the feasibility of the classification method derived in our previous study[6].

MATERIALS AND METHODS

I. *Herb Materials*

Twenty-eight herb samples were collected from different herb shops both in Taiwan and in Mainland China. Samples 4, 7, 9, 10, 22, 23, 24 were obtained from Taipei, Taiwan. Those collected in Mainland China include samples 3, 21, 26-28 from Sichuan Province, samples 14, 16 from Guichou Province, and samples 19, 20 from Yunnan Province. The others were provided by Brion Research Institute of Taiwan. These samples were identified by external appearance and categorized by histological anatomy into 4 origins: **I** (samples 11-13), **III** (samples 3-10), **IV** (samples 1, 2), and **V** (samples 14-28), respectively.

II. *Standards*

Berberine chloride was purchased from Sigma (St. Louis, MO, USA). Brucine was purchased from Merck (Dermstadt, Germany). Palmatine, phellodendrine, and magnoflorine were isolated from Phellodendri Cortex[3]; jatrorrhizine was isolated from Coptidis Rhizoma[8].

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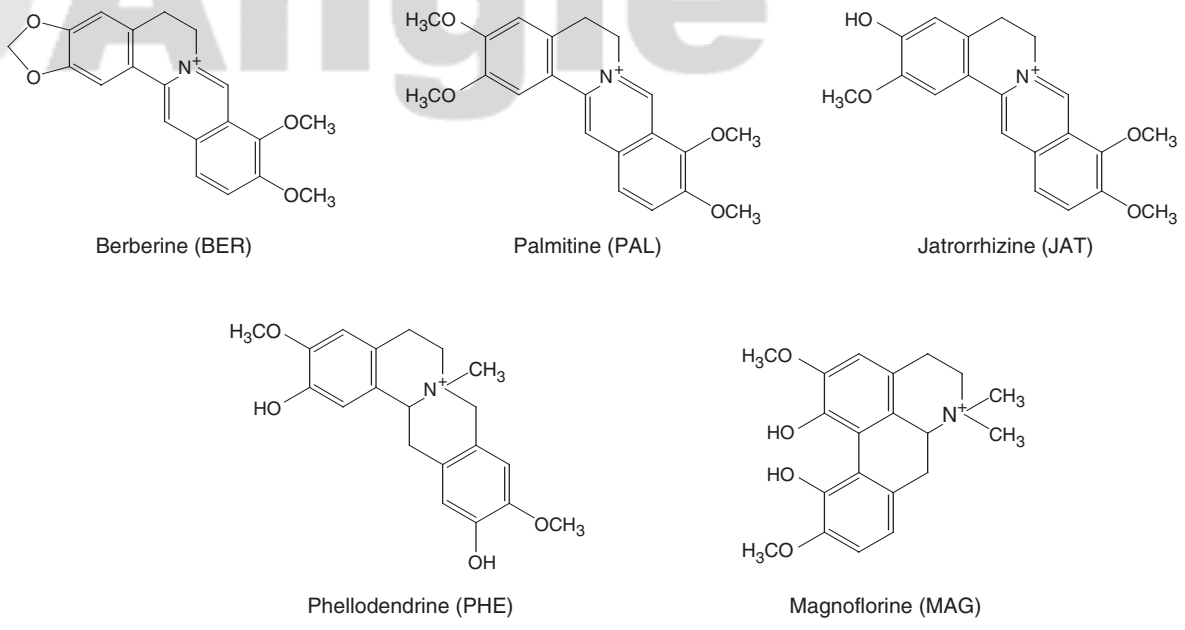


Figure 1. Chemical structures of alkaloids in Phellodendri Cortex.

Table 1. Major characteristics of various species of *Phellodendron*

Species / Item	*P. wilsonii* **I**	*P. amurense* var. *sachalinense* **II**	*P. amurense* **III**	*P. chinense* **IV**	*P. chinense* var. *glabrius culum* **V**
Fibers of ground cortex	Fine and short, dark brown	Fine and short, golden brown	Fine and short, some are flaky light brown	Fine threads, light yellow	Coarse and flaky, bright yellow to light brown
Fracture surface	Golden brown	Bright to deep yellow	Yellow-green	Light yellow	Bright yellow or golden brown
Cortical stone cells	Mostly in long stick form, comprising several thin-membraned stone cells	Solitary in global form, or several ones forming a long stick form	Solitary or clustered, irregular long stick form	Solitary or clustered, irregular long stick form	Solitary long stick form, up to 300~900 μm

III. *Sample Preparation*

A 0.5 g sample of pulverized Phellodendri Cortex was extracted with 7.0 mL of 70% methanol by stirring at room temperature for 30 min and then by centrifuging at 1500 ×g for 10 min. The extraction was repeated three times, and the aliquots were combined. After an addition of 2.5 mL of internal standard solution (2 mg brucine in 1 mL of 70% methanol), the total volume of Phellodendri Cortex extract was brought to 25 mL with 70% methanol. This solution was passed through a 0.45 μm filter and the filtrate was injected (5s electrodynamic at 5 kV) into the capillary electrophoresis system directly. The area ratios of the absorption peaks were then calculated.

IV. *Apparatus and Conditions*

All analyses were carried out on an HP^{3D} Capillary Electrophoresis System equipped with a photodiode array detector set at 280 nm and a 50 cm × 50 μm i.d. uncoated capillary (Polymicro Technologies, Phoenix, USA) with the detection window placed at 41.5 cm. The conditions were identical to those of Liu's study[6]; i.e. the applied voltage was 15 kV (constant voltage, positive to negative polarity) and the temperature was 25 ± 0.5˚C. The electrolyte was a buffer solution consisting of 50% of 0.5 M sodium acetate solution (pH = 4.6, adjusted with acetic acid) and 50% of acetonitrile. The electrolyte was filtered through a 0.45 μm PVDF filter before use.

RESULTS AND DISCUSSION

By carefully inspecting the features of external appearances and histological anatomy of the samples with those given in references[7-13], the 28 samples were identified to originate from 4 species: 2 samples of *Phellodendron chinense* (**IV**), 8 samples of *P. amurense* (**III**), 3 samples of *P. wilsonii* (**I**), and the other 15 samples of *P. chinense* var. *glabrius culum* (**V**), and the last species was of special interest in this study. The major characteristics of various species are described in Table 1. The characteristics, such as the coarse and flaky fibers for **V**, the green color of fracture sur-

faces of **III**, and the typical thin and long stone cells of **V** which can be up to 300~900 μm of length were observed. Analytical results of these commercial samples with capillary electrophoresis are shown in Tables 2, and the data of 31 samples from Liu's study[6] are also included to make a detailed comparison and to reach a final conclusion.

Comparing the electropherograms of **I**, **III**, and **V** (Figure 2A~2C) with those in our previous study, two basic changes can be observed: the total migration time lagged 3 min (8 min to 11 min), and the migration order of MAG and brucine (internal standard, IS) was switched which might be due to the different capillaries, chemicals, or analytical instruments used. Since the quantitative data were calculated from the area ratio of individual alkaloid to that of IS, the analytical results should not be significantly affected by these differences. It was also noted that

Table 2. Alkaloid contents in the commercial Phellodendri Cortex samples (mg/g)

Species	sample	BER	PAL	JAT	PHE	MAG	TA	TA/ BER	TA/ MAG
P. chinense	1	3.3	5.4	0.2	0.8	3.6	13.2	4.1	3.7
IV	2	6.1	4.0	0.2	1.1	5.7	17.0	2.8	3.0
	Mean (n = 2)	4.7	4.7	0.2	1.0	4.7	15.1	3.4	3.3
	± SD	2.0	1.0	0.0	0.2	1.5	2.7	0.9	0.5
	(I) mean (n = 14)	5.7	3.4	0.2	1.0	5.2	15.4	3.0	3.4
	± SD	2.8	2.0	0.1	0.3	2.3	6.0	0.9	1.8
	(II) mean (n = 16)	5.6	3.6	0.2	1.0	5.1	15.4	3.1	3.4
	± SD	2.7	1.9	0.1	0.3	2.1	5.6	0.8	1.7
P. amurense	3	3.6	2.3	0.2	0.5	1.9	8.4	2.3	4.5
III	4	3.9	2.6	0.2	1.1	4.1	11.8	3.0	2.9
	5	3.9	2.5	0.2	0.9	3.7	11.2	2.9	3.0
	6	4.5	2.1	0.2	0.7	3.5	11.0	2.5	3.1
	7	4.7	2.5	0.2	0.9	4.0	12.3	2.6	3.1
	8	6.1	2.6	0.3	1.1	6.1	16.2	2.6	2.7
	9	6.6	2.3	0.5	2.4	6.3	18.1	2.7	2.9
	10	6.9	2.5	0.2	1.3	9.0	19.8	2.9	2.2
	Mean (n = 8)	5.0	2.4	0.2	1.1	4.8	13.6	2.7	3.0
	± SD	1.3	0.2	0.1	0.6	2.2	4.0	0.2	0.7
	(I) mean (n = 4)	6.2	3.3	0.2	1.1	4.7	15.5	2.5	5.1
	± SD	2.3	1.1	0.1	0.4	2.9	6.2	0.6	3.7
	(II) mean (n = 12)	5.4	2.7	0.2	1.1	4.8	14.2	2.6	3.7
	± SD	1.8	0.8	0.1	0.5	2.5	5.0	0.4	2.5
P. wilsonii	11	30.2	1.2	0.5	2.5	2.7	37.1	1.2	13.7
I	12	29.8	0.7	0.5	2.5	1.9	35.4	1.2	19.1
	13	36.6	0.5	0.3	3.1	1.4	41.8	1.1	29.5
	mean (n = 3)	32.2	0.8	0.4	2.7	2.0	38.1	1.2	20.8
	± SD	3.8	0.4	0.1	0.3	0.7	3.4	0.0	8.0
	(I) mean (n = 7)	35.2	0.8	0.5	2.9	1.6	41.0	1.2	31.0
	± SD	6.8	0.7	0.2	0.4	1.0	7.8	0.0	14.3
	(II) mean (n = 10)	34.3	0.8	0.4	2.8	1.7	40.2	1.2	27.9
	± SD	6.0	0.6	0.2	0.4	0.9	6.8	0.0	13.2
P. amurense var.	(I) mean (n = 6)	31.7	1.9	0.7	2.6	5.1	41.8	1.3	11.7
sachalinense **II**	± SD	6.6	1.4	0.4	0.8	3.6	10.3	0.2	7.6
P. chinense	14	19.4	8.0	0.6	2.4	4.5	34.8	1.8	7.7
var. *glabrius culum*	15	20.7	4.8	0.6	2.0	2.5	30.6	1.5	12.4
V	16	19.1	2.5	0.4	1.6	2.2	25.9	1.4	11.8
	17	16.7	1.5	0.2	1.4	3.2	23.0	1.4	7.1
	18	16.5	1.1	0.1	1.6	3.0	22.3	1.4	7.3
	19	17.0	0.7	0.4	1.4	1.0	20.5	1.2	19.9
	20	15.8	0.6	0.3	1.3	0.7	18.7	1.2	26.8
	21	23.2	0.7	0.2	2.0	3.5	29.7	1.3	8.4
	22	20.2	0.5	0.3	2.3	0.4	23.7	1.2	55.1
	23	17.0	0.3	0.2	1.9	1.7	21.0	1.2	12.6
	24	22.2	0.2	0.2	1.9	0.6	25.1	1.1	41.2
	25	26.4	0.2	0.1	2.0	1.1	29.7	1.1	27.0
	26	32.4	0.2	0.1	2.1	0.3	35.1	1.1	125.2
	27	16.6	0.1	0.1	1.4	0.6	18.8	1.1	31.9
	28	34.7	0.1	0.1	2.2	0.2	37.3	1.1	169.6
	Mean (n = 15)	21.2	1.4	0.3	1.8	1.7	26.4	1.3	37.6
	± SD	5.8	2.2	0.2	0.3	1.4	6.1	0.2	47.5

(I): data from Liu's report[6].

(II): data of total samples from both studies.

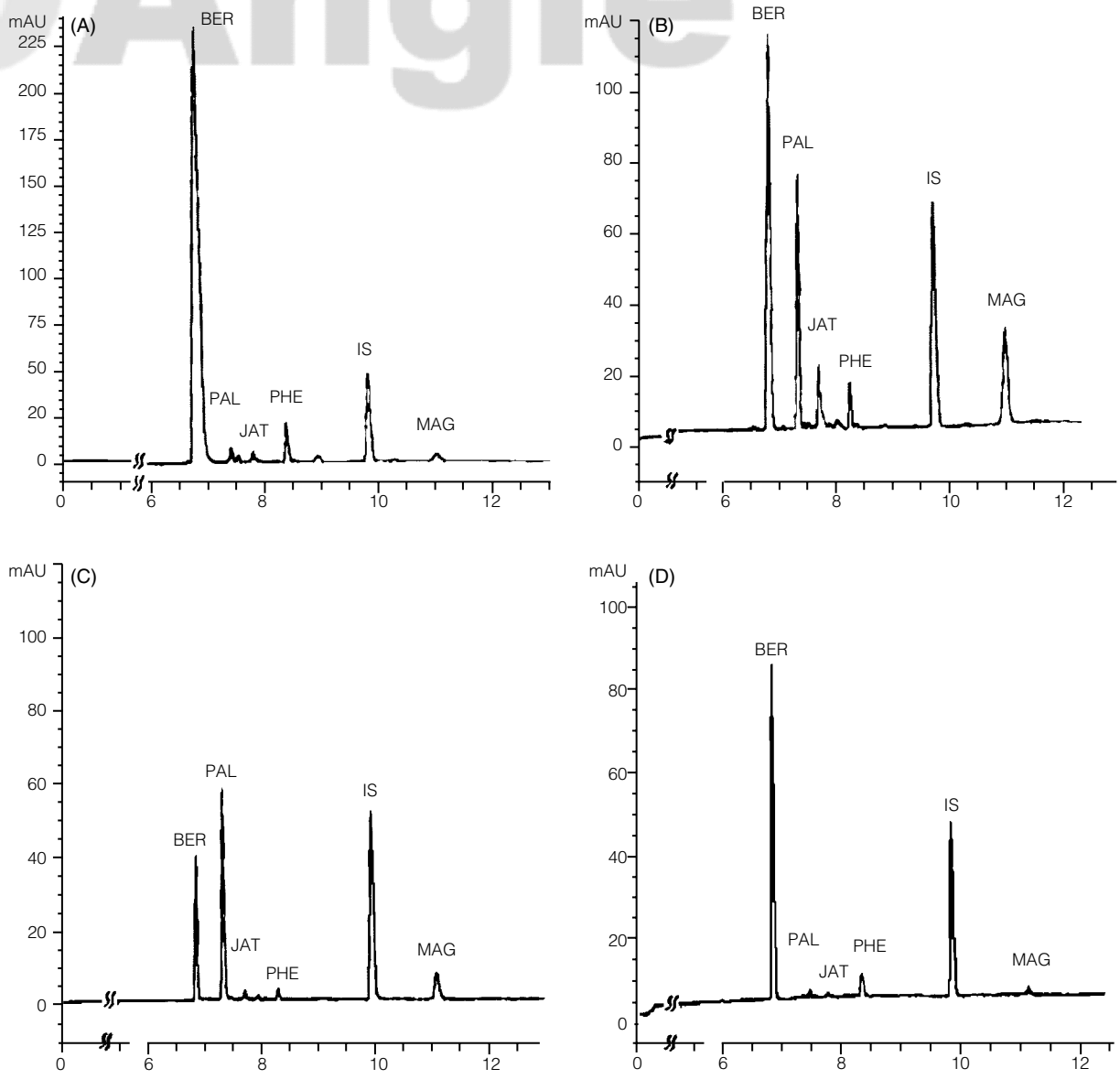
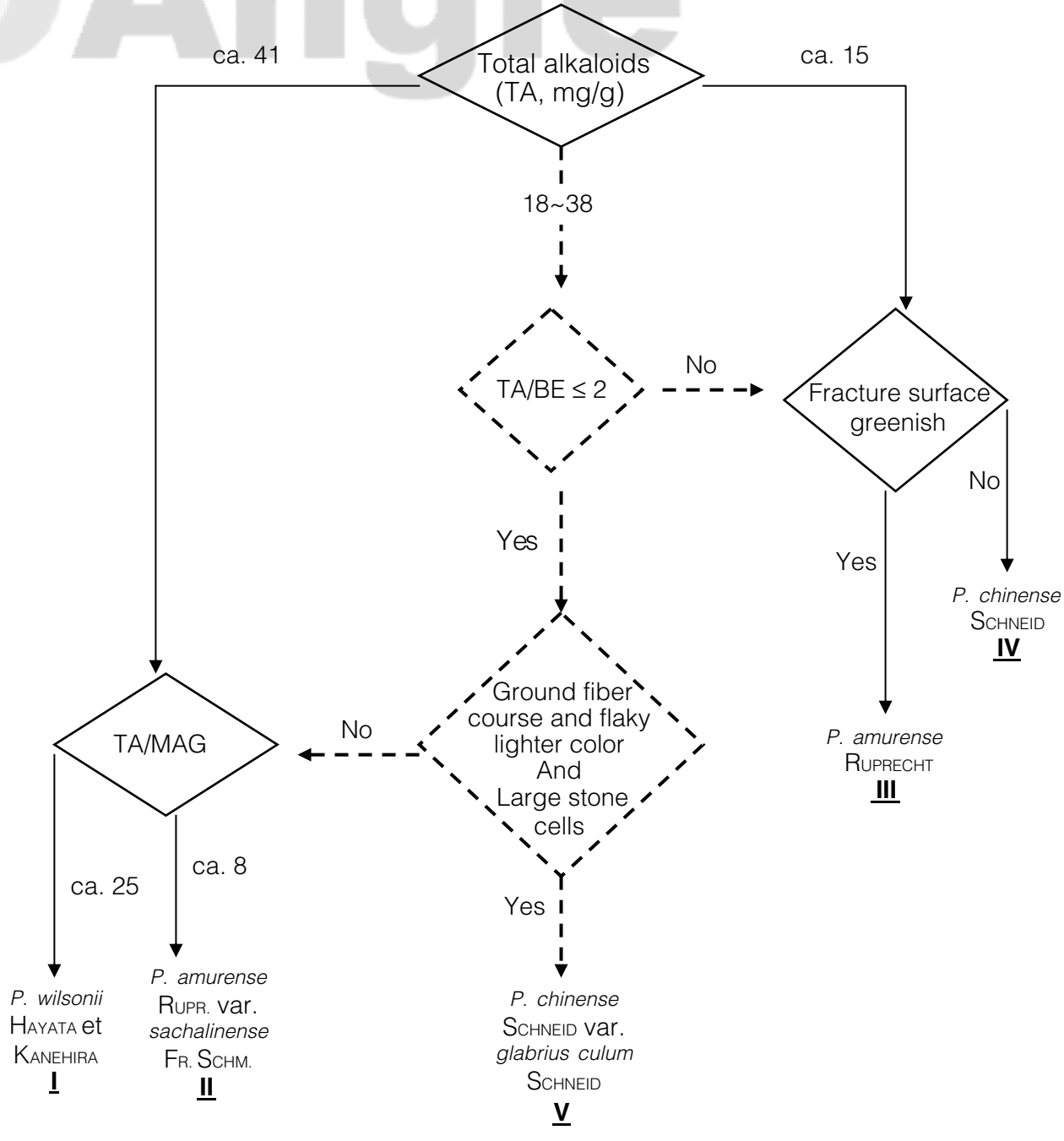


Figure 2. Capillary electropherograms of the extracts of Phellodendri Cortex (A): *P. wilsonii* (sample 13); (B): *P. amurense* (sample 8); (C): *P. chinense* (sample 1); (D): *P. chinense* var. *glabrius culum* (sample 16). The abbreviations are as follows: BER for berberine, PAL for palmitine, JAT for jatrorrhizine, PHE for phellodendrine, and MAG for magnoflorine. IS stands for an internal standard (burcine).

samples derived from the same origin had almost identical patterns: BER content is the highest in almost all species except samples of **III** and **IV**, in which MAG were comparable to BER. On the other hand, PHE was the second major components for **I** and **II** but their contents were not as high as in **III** and **IV**. For **V**, more samples have patterns similar to **I**, as shown in Figure 2D, but the differences among samples are diversified to a greater extent than those of the other species.

The statistical data of the 28 samples collected in this study is very consistent to that of the 31 samples in our previous study as shown in Table 2. As deduced from Liu's report, the most marked distinction is the content of total alkaloids (TA) and the quotient of total alkaloids divided by magnoflorine (TA/MAG). In regard to TA contents, species I (40.2 ± 6.8 mg/g, n = 10) and **II** (41.8 ± 10.3 mg/g, n = 6) are superior to the other group of **III** (14.2 ± 5.0 mg/g, n = 12) and **IV** (15.4 ± 5.6 mg/g, n = 16), while the major origin of Phellodendri Cortex samples in the current market is **V** which has the TA contents in the range of 18~38 mg/g (n = 15). The quotient of TA/MAG is useful in distinguishing **I** (ca. 25) from **II** (ca. 8) in the previous report, and the appropriate cutoff value can be found to be ca. 20. Further investigation on the alkaloid contents revealed another quotient of TA/BER to be useful. The value is less than 2 (ca. 1.3) for species **I**, **II**, and **V** because berberine is the major alkaloid; whereas the value is greater than 2 (ca. 3) for species **III** and **IV** in which the contents of palmatine and magnoflorine are comparable to that of berberine.

Accordingly, the 5 origins of Phellodendri Cortex can



Note: Solid lines represent the rules derived by Liu in previous study, and the dotted lines were the rules derived in this study for **V**.

Figure 3. The flow chart for identifying the origins of Phellodendri Cortex.

be easily identified by chemical analysis and external features, and the classification methods are represented in the flow chart and in Figure 3. In the beginning of the flow chart, the value of total alkaloids is regarded as not only an assessment of quality but also a guideline for species distinction. A sample with TA in the range of 18 to 38 mg/g can be firstly classified as **V**, those with higher values are **I** and **II**, and lower values are categorized as **III** and **IV**. Secondly, **I** and **II** are further distinguished by the quotient of TA/MAG, while **III** and **IV** can be distinguished by external appearances, such as fracturability and color of fracture surface. As for some exceptions of the ambiguous samples, for instance, **III** and **IV** of higher TA contents (TA > 18 mg/g, e.g. samples 9, 10) could be classified as **V** firstly but would be returned at the second decision box because their TA/BER is greater than 2. The hardest case

was when inferior samples of **I** and **II** (TA < 38 mg/g, e.g. samples 11, 12) are classified as **V** or vice versa, their ground fibers could serve as a good source for identification. The ground fibers of **I** and **II** are fine and short with darker colors; while **V** is coarse and flaky with lighter colors (Table 1). Otherwise, a most reliable way to identify its origin would be the observation of stone cells since it is a typical characteristic of **V** (Table 1).

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