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The Use of Lobetyolin and HPLC-UV Fingerprints for Quality Assessment of Radix Codonopsis

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ABSTRACT

In 2005 edition of the Chinese Pharmacopoeia, lobetyolin was used as a marker for TLC identification of Radix Codonopsis, whose source plants are *Codonopsis pilosula*, *C. pilosula* var. *modesta* and *C. tangshen*. Quantification of lobetyolin in 44 samples derived from eight species of *Codonopsis* and another four genera of the same family Campanulaceae revealed that lobetyolin is present in all *Codonopsis* species and in *Campanumoea javanica*, *Platycodon grandiflorum* and *Lobelia chinensis*. In fact, the other five *Codonopsis* species are commonly used as the substitutes of Radix Codonopsis, and the roots of *Campanumoea javanica* and *Platycodon grandiflorum* are sometimes found as the adulterants. Contents of lobetyolin were found variable among different samples, and some substitutes and adulterants actually had comparably high contents of lobetyolin. Therefore, lobetyolin should be taken only as a general but not a definitive marker for the identification of Radix Codonopsis. HPLC-UV fingerprints with seven characteristic peaks were established for discriminating Radix Codonopsis from its adulterants, but more specific and definitive markers remain to be identified for differentiating Radix Codonopsis from its substitutes.

Key words: Radix Codonopsis, lobetyolin, quantification, HPLC, fingerprint

INTRODUCTION

Radix Codonopsis is a famous traditional Chinese medicine used for replenishing energy deficiency, strengthening the immune system, lowering blood pressure and improving appetite[(1)]. It is often regarded as a tonic equivalent to ginseng. Experimental bioassays have demonstrated that the extracts of Radix Codonopsis have beneficial effects to relieve gastrointestinal motility disorders[(2)], contribute to the immunoregulatory functions[(3)] and enhance learning and memory[(4)].

According to the Chinese Pharmacopoeia, the roots of *Codonopsis pilosula* (Franch.) Nannf., *C. pilosula* (Franch.) Nannf. var. *modesta* (Nannf.) L. T. Shen and *C. tangshen* Oliv. are regarded as the genuine source for Radix Codonopsis[(5)]. It has also been reported that the roots of some *Codonopsis* species, such as *C. tubulosa* Kom., *C. subglobosa* W. W. Smith, *C. clematidea* (Schynek) C. B. Cl., *C. canescens* Nannf., and *C. lanceolata* (Sieb. et Zucc.) Trautv. are used as the substitutes of Radix Codonopsis at local producing areas in China. Furthermore, the roots of *Campanumoea javanica* Bl. and *Platycodon grandiflorum* (Jacq.) A. DC. from the same family Campanulaceae, are sometimes found as the adulterants of Radix Codonopsis[(6,7)]. Therefore, authentication and quality control are very important for the quality assurance of Radix Codonopsis.

The roots of *Codonopsis* species contain polysaccharides, triterpenes, phytosterols, sesquiterpenes, phenolic glycosides, alkaloids and polyacetylenes[(8-20)]. Among these compounds, a polyacetylene called lobetyolin has been reported to have protective properties against injuries of the gastric mucous membrane induced by ethanol[(21)]. Lobetyolin was used as a marker for TLC identification of Radix Codonopsis in the Chinese Pharmacopoeia (2005 edition)[(5)].

This study looks into the value of lobetyolin as a chemical marker for Radix Codonopsis, by checking the occurrence and content of this component in eight *Codonopsis* species and another four genera of Campanulaceae. An HPLC-UV fingerprint method was also explored for the authentication of Radix Codonopsis.

MATERIALS AND METHODS

I. *Chromatography*

Quantitative and fingerprint analysis were performed on an Agilent 1100 chromatography system with DAD detector. A Zorbax XDB RP-C18 column

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(4.6 × 250 mm, 5 μm, Agilent Technologies, USA) and a RP-C18 guard column were used. The column temperature was set at 20°C. The mobile phase consisted of acetonitrile and water. Linear gradient elution from 10% to 40%, and then to 100% acetonitrile respectively in 25 min and 35 min were applied. The detection wavelengths were at 267 and 295 nm.

II. *Reagents*

HPLC grade acetonitrile and ACS grade methanol were purchased from International Laboratory (Nevada, USA). HPLC grade water was prepared with Millipore Milli-Q SP water purification system. Lobetyolin was isolated and purified from the roots of *C. tubulosa* by adsorbent resins (Diaion®HP-20) and silica gel chromatography. Its purity was determined to be higher than 98% by HPLC analysis, and its identity was confirmed by MS and NMR analyses. Lobetyolinin was a gift from Prof. Kanji Ishimaru (Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Japan) (Figure 1).

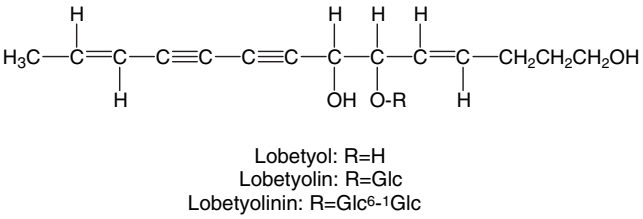

Figure 1. Chemical structures of polyacetylenes.

III. *Materials*

A total of 44 samples from Family Campanulaceae were collected from different regions in China (Table 1). The species were identified carefully by morphological and anatomical characteristics as well as DNA analysis[7,22]. Voucher samples were deposited in the laboratory of Hong Kong Jockey Club Institute of Chinese Medicine and the Museum of the Institute of Chinese Medicine, the Chinese University of Hong Kong.

IV. *Sample Preparation Procedures*

Samples were pulverized and the powder was screened through 180 μm sieves. Fine powder (1 g) was accurately weighed, 25 mL of methanol was added and the mixture was weighed again. The powder was then extracted by refluxing for 2 hr. After cooling, methanol was added to make up to the initial weight. The supernatant was filtered through a syringe filter (0.45 μm). Ten microliter of each filtrate was subject to HPLC analysis.

V. *Validation of Lobetyolin Quantification*

Lobetyolin was weighed, dissolved and diluted with methanol in a volumetric flask to obtain standard solutions for the calibration curve. The calibration curve was constructed by plotting peak area (Y) versus the amount of analyte (X), and expressed as the following equation: $Y = 828.6\ X + 0.5645$ ($r^2 = 0.9999$). The test range of calibration curve was 7.2-720.0 ng (n = 8). The contents of lobetyolin in samples were calculated using the calibration curve.

The sample of Radix Codonopsis (No. 21) was extracted and processed in duplicate according to the procedures above. The procedure was repeated five times to evaluate the reproducibility of extraction protocol. The relative standard deviation was 1.15%, indicating satisfactory reproducibility.

Quintuplicate samples of Radix Codonopsis (No. 21) were accurately spiked with known amount of lobetyolin (775 mg/each), and then extracted and analyzed. Good recovery from the analysis was obtained as 97.2% to 101.6% for lobetyolin.

RESULTS AND DISCUSSION

I. *Quantitative Analysis*

Totally 44 samples derived from 12 species were analyzed under the present conditions. The representative chromatograms monitored at 267 nm and 295 nm are shown in Figure 2. The identity of lobetyolin (**1**) in samples was confirmed by comparing its retention time and online spectrum with that of the reference compound. The contents of lobetyolin in 44 samples are shown in Table 1.

The results showed that lobetyolin was detected not only in the three *Codonopsis* species used for Radix Codonopsis, but also in other species of the same genus, including *C. tubulosa*, *C. subglobosa*, *C. clematidea*, *C. canescens* and *C. lanceolata*, which are common commercial substitutes of Radix Codonopsis. Moreover, lobetyolin was also found in other genera from the family Campanulaceae, such as *Campanumoea javanica*, *Platycodon grandiflorum* and *Lobelia chinensis*; the former two species are occasionally found in the herb market as adulterants of Radix Codonopsis. Under the same condition, lobetyolin was not detected only in *Adenophora tetraphylla* among the 12 species analyzed.

Variable amount of lobetyolin ranging from 0.010 mg/g to 0.816 mg/g were determined in the three species used for Radix Codonopsis. Different contents ranging from 0.019 mg/g to 0.794 mg/g were also found in the other species of *Codonopsis* analyzed. In addition, this compound was determined with relatively high content in some species from other genera in family Campanulaceae, such as *Campanumoea javanica* (0.200 mg/g) and *Lobelia chinensis* (0.320-0.525 mg/g), while *Platycodon grandiflo-*

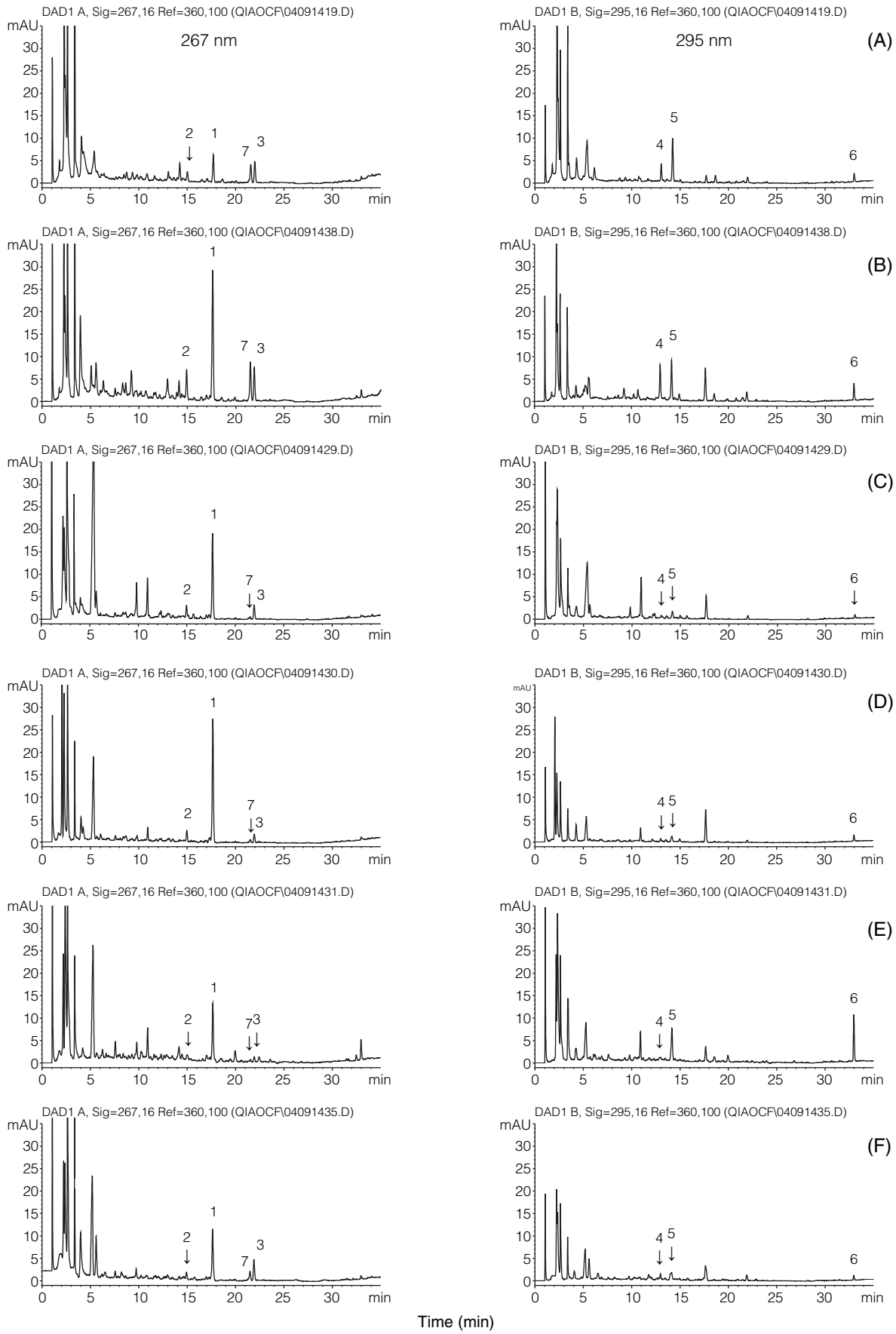

Figure 2. Representative HPLC chromatograms of Radix Codonopsis and related species. (A) *Codonopsis pilosula*; (B) *C. pilosula* var. *modesta*; (C) *C. tangshen*; (D) *C. tubulosa*; (E) *C. subglobosa*; (F) *C. clematidea*.

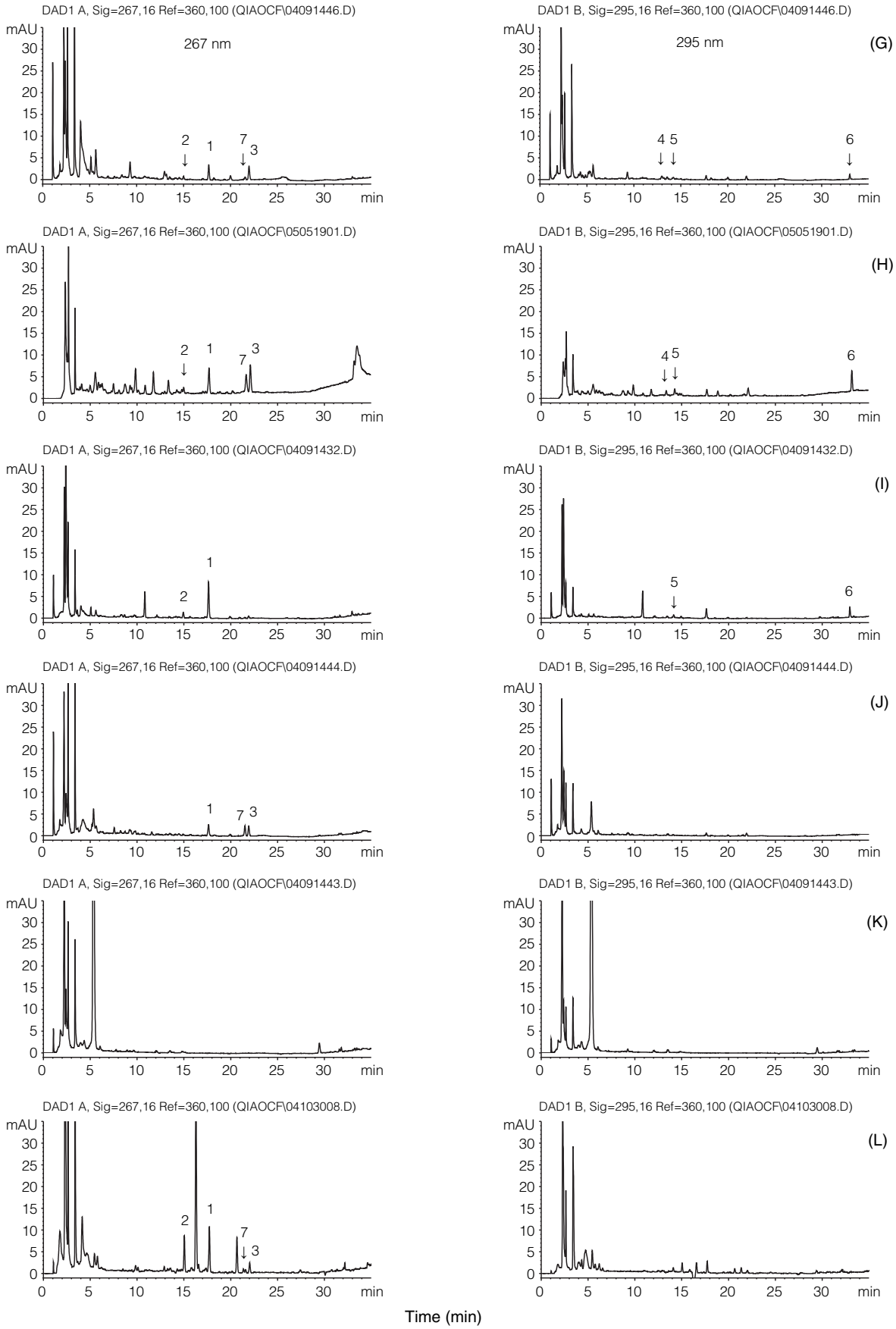

Figure 2. Representative HPLC chromatograms of Radix Codonopsis and related species (continued). (G) *C. canescens*; (H) *C. lanceolata*; (I) *Campanumoea javanica*; (J) *Platycodon grandiflorum*; (K) *Adenophora tetraphylla*; (L) *Lobelia chinensis*.

Table 1. Contents of lobetyolin in Radix Codonopsis and related species

Sample No.	Species	Source	Lobetyolin (mg/g)
1	*Codonopsis pilosula*	Gansu	0.013
2		Jilin	0.017
3		Henan	0.011
4		Hebei	0.010
5		Jilin, Jilin	0.032
6		Dunhua, Jilin	0.144
7		Jinzhou, Liaoning	0.030
8		Changchun, Jilin	0.011
9		Pingshun, Shanxi	0.261
10		Pingshun, Shanxi	0.239
11		Shenzhen, Guangdong*	0.083
12		Shuozhou, Shanxi*	0.041
13		Shuozhou, Shanxi*	0.166
14		Shuozhou, Shanxi*	0.118
15		Shuozhou, Shanxi*	0.019
16		Shuozhou, Shanxi*	0.099
17	*C. pilosula* var. *modesta*	Sichuan	0.220
18		Yunnan	0.163
19		Nanbu, Sichuan	0.030
20		Wenxian, Gansu	0.064
21		Hong Kong*	0.816
22		Hong Kong*	0.340
23		Hong Kong*	0.341
24		Hong Kong*	0.582
25		Shenzhen, Guangdong*	0.469
26		Guangzhou, Guangdong*	0.067
27	*C. tangshen*	Yunnan	0.256
28		Wuxi, Chongqing	0.492
29	*C. tubulosa*	Xichang, Sichuan	0.714
30	*C. subglobosa*	Ganzi, Sichuan	0.351
31		Kunming, Yunnan	0.070
32	*C. clematidea*	Wulumuqi, Xinjiang	0.336
33		Xinjiang	0.277
34		Xinjiang	0.325
35	*C. canescens*	Liangshan, Sichuan	0.019
36		Kunming, Yunnan	0.062
37	*C. lanceolata*	Hong Kong	0.794
38		Guangzhou, Guangdong*	0.180
39	*Campanumoea javanica*	Emeishan, Sichuan	0.200
40	*Platycodon grandiflorum*	Hubei	0.070
41	*Adenophora tetraphylla*	Hong Kong*	-**
42		Beijing*	-
43	*Lobelia chinensis*	Shenzhen, Guangdong*	0.525
44		Hong Kong*	0.320

*Commercial sample.
**Not detected.

rum contains relatively lower content of 0.070 mg/g. Therefore, no significant correlations can be made concerning the content of lobetyolin among the genuine sources of Radix Codonopsis and its substitutes or adulterants.

Based on the above results, lobetyolin should be taken only as a general but not a definitive chemical marker for the identification of Radix Codonopsis.

II. *HPLC Fingerprint Analysis*

Since lobetyolin is widely found in the various species, it is not appropriate to take it as a single marker. Thus HPLC fingerprint was used to evaluate Radix Codonopsis.

Besides lobetyolin, another principal polyacetylenic glucoside was identified as lobetyolinin (**2**) by comparing with the corresponding retention time and the UV spectra of the reference compound. These two polyacetylenic glucosides displayed a uniform and unique type of UV spectra which showed a characteristic palm-like (or sawtooth-like) shape between 225-300 nm with maximum absorption (λ_{max}) at 267 nm (Figure 3). With the same UV spectra and longer retention time, compound **3** was suspected to possess the same structural skeleton and presumed to be lobetyol (Figure 1) which is the aglucone of lobetyolin and lobetyolinin. With similar UV spectra to peak **1-3**, another three peaks at 295 nm including **4-6** were found in the HPLC chromatograms of Radix Codonopsis. The UV spectra of **4-6** showed the characteristic palm-like (or sawtooth-like) shape too, but the absorption shifted to 250-325 nm with λ_{max} at 295 nm (Figure 3). With the unique UV characteristic, they were presumed to be other polyacetylenic compounds. Besides these six peaks, peak **7** was also found to exist in the HPLC chromatograms from many samples. Unlike the above two types, the spectrum of peak **7** showed obvious absorption between 225-300 nm with the λ_{max} at 263 nm (Figure 3).

Based on the HPLC chromatograms of the 44 samples, seven peaks were selected as characteristic peaks, and their retention times are 17.62 min (**1**), 14.92 min (**2**), 21.91 min (**3**), 12.96 min (**4**), 14.14 min (**5**), 32.96 min (**6**) and 21.51 min (**7**), respectively.

On the basis of the established chromatographic profiles, the analytical results of 44 samples from 12 related species were compared with each other. The samples from the genus *Codonopsis*, including the species used as Radix Codonopsis and five substitutes, contain all seven peaks belonging to three types. In contrast, peaks **3**, **4** and **7** were absent in *Campanumoea javanica*, peaks **4-6** (type-II) were missing in *Platycodon grandiflorum* and *Lobelia chinensis*. Furthermore, none of the seven characteristic components was detected in *Adenophora tetraphylla*. These results suggested that the HPLC fingerprints could help distinguishing samples of the genus *Codonopsis* from those of the other four genera tested.

In conclusion, lobetyolin should be taken only as a general reference for Radix Codonopsis. HPLC-UV fingerprints could help distinguishing Radix Codonopsis from its common adulterants. More specific and definitive markers remain to be identified for discriminating Radix Codonopsis from its substitutes.

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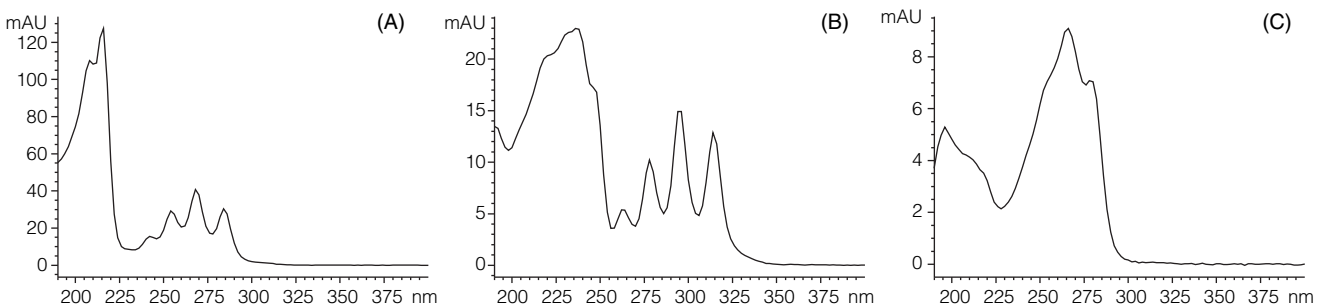

Figure 3. UV spectra of the seven selected peaks. (A) Peak 1-3; (B) Peak 4-6; (C) Peak 7.

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