

Authentication and Quantitative Analysis on the Chemical Profile of Cassia Bark (Cortex Cinnamomi) by High-Pressure Liquid Chromatography

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Cassia bark or cortex cinnamomi, the dried stem bark of *Cinnamomum cassia* Presl. (Lauraceae), is a popular natural spice and a commonly used herb in traditional Chinese medicine. However, adulterants are frequently found in the market. In this study, 44 samples of Cassia bark including bark from seven related *Cinnamomum* species were collected from fields and market. Four characteristic components, cinnamaldehyde, cinnamic acid, cinnamyl alcohol, and coumarin were determined by RP-HPLC, and a fingerprint comprised of five markers was established. These results showed that cassia barks contained high contents of cinnamaldehyde (13.01–56.93 mg/g). The highest content of cinnamaldehyde (up to 93.83 mg/g) was found in debarked cortex, which is traditionally regarded as having the best quality in local herb shops. In contrast, the adulterants from the other *Cinnamomum* species, *C. wilsonii* Camble, *C. japonicum* Sieb., *C. mairei* Levl. and *C. burmanii* (Nees) Blume, contained low contents of cinnamaldehyde (<2.00 mg/g). The content of cinnamaldehyde in *C. loureirii* Nees was comparable to that in *C. cassia*. It is suggested that five characteristic peaks by HPLC are suitable for distinguishing genuine cassia bark from the adulterants and could be applied in the quality control of this commodity.

KEYWORDS: Cortex cinnamomi; *Cinnamomum*; *Cinnamomum cassia*; cinnamaldehyde; quantitative analysis; chemical profile; HPLC fingerprint

INTRODUCTION

Cassia bark refers to the bark of *Cinnamomum cassia* Presl. (Lauraceae). It is commonly used as traditional Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases (1). It is also used as a popular natural spice in many parts of the world. It is scheduled as cortex cinnamomi in the Chinese pharmacopoeia (2). However, barks from other *Cinnamomum* species are frequently found as substitutes or adulterants (1). The use of other *Cinnamomum* species may significantly affect its intended therapeutic values.

Various aromatics, diterpenes, and polyphenols have been identified in cassia bark (3–6). Li *et al.* (7) found that 67.21%

of volatile oil in the bark was identified as cinnamaldehyde by GC–MS. Also, similar studies showed the volatile oil of cassia bark and cassia twig (ramulus cinnamomi) contained 83.10 and 64.57% cinnamaldehyde, respectively (8). Cinnamaldehyde is one of the active ingredients of cassia bark. It was reported that cinnamaldehyde inhibited the production of lymphocytes and modulated T-cell differentiation (9). In addition, it exhibited antifungal, cytotoxic, antipyretic, antioxidant, antimicrobial, and mosquito larvicidal effects (10–15).

Previous studies on *Cinnamomum* species with TLC, GC, GC–MS, and HPLC are well-documented (1, 7, 8, 16–23). However, a systematic study on the authentication and assessment on related *Cinnamomum* species is not well-defined. In this study, samples of cassia bark and related materials from different regions as well as four types of commercial commodities (debarked cortex, Qi-Bian-Gui, bark and twigs) sold in Hong Kong were collected and analyzed. The main chemical components including cinnamaldehyde, cinnamic acid, cinnamyl alcohol, and coumarin were selected as markers for quantitative analysis. Detailed results on HPLC quantitative analysis and

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the fingerprints were established that could be employed for authenticating genuine cassia bark and assessing its quality efficiently.

MATERIALS AND METHODS

Chromatography. Quantitative analyses were performed on an Agilent 1100 chromatography system with DAD detector. A 4.6 × 150 mm, 5 μm, Zorbax XDB RP-C₈ column (Agilent Technologies) together with a RP-C₁₈ guard column were used with column temperature set at 20 °C. The mobile phase consisted of acetonitrile and 0.04% aqueous acetic acid (25:75), and the flow rate was 1.0 mL/min. The detection wavelengths were at 280 nm for cinnamaldehyde, cinnamic acid, and coumarin and at 250 nm for cinnamyl alcohol, respectively.

The chemical fingerprint analyses were performed under the same conditions. The mobile phase consisted of acetonitrile (eluent A) and 0.02% aqueous acetic acid (eluent B). Linear gradient elution from 10 to 50% eluent A in 60 min was applied. The detection wavelength was at 280 nm.

Reagents. HPLC-grade acetonitrile and ACS-grade methanol were purchased from International Laboratory (NV). HPLC-grade acetic acid was purchased from BDH Laboratory Supplies (Poole, U.K.). HPLC water was prepared with the Millipore Milli-Q SP water purification system. Cinnamaldehyde was isolated and purified from the bark of authentic *C. cassia* by silica gel chromatography eluted with hexane/acetone (10:1). The purified cinnamaldehyde was kept at 4 °C. Its purity was determined to be higher than 98% by HPLC analysis, and its structure was confirmed by NMR analysis. Cinnamyl alcohol and eugenol were purchased from Acros Organics (NJ). Coumarin was purchased from Sigma (St. Louis, MO). Cinnamic acid was supplied by the Chinese National Institute for the Control of Pharmaceutical and Biological Products.

Materials. A total of 13 samples of the bark from *C. cassia*, 16 samples from 6 other *Cinnamomum* species, and 15 different market commodities of Cassia bark and related materials were collected from China, Vietnam, and Korea (Tables 1 and 2). The species of genus *Cinnamomum* were identified carefully by morphological characteristics. Voucher samples were deposited in the laboratory of the Hong Kong Jockey Club Institute of Chinese Medicine and at the Museum of the Institute of Chinese Medicine, Chinese University of Hong Kong.

Sample Preparation Procedures. Samples were pulverized, and the powder was screened through 180 μm sieves. Fine powder (0.5 g) was accurately weighed, and 25 mL of methanol was added and the mixture was weighed again. Then, the powder was extracted by ultrasonication at room temperature for 30 min. After cooling, methanol was added to make up to the initial weight. The supernatant fluid was filtered through a syringe filter (0.45 μm). A total of 0.5–10 μL (adjusting the volume according to the concentration) of each filtrate was subject to HPLC quantitative analysis, and 1 μL was subject to chemical-profiling analysis.

Preparation of the Calibration Curves. Cinnamaldehyde, cinnamic acid, cinnamyl alcohol, and coumarin were weighed, dissolved, and diluted with methanol in a volumetric flask to obtain standard solutions for the calibration curves. The ranges of calibration curves were 7.58–947.5, 0.119–23.8, 0.165–33.0, and 0.171–342 ng, respectively ($n = 7$). The contents of cinnamaldehyde, cinnamic acid, cinnamyl alcohol, and coumarin were calculated using the respective calibration curves.

RESULTS AND DISCUSSION

Collection of Samples. A total of 29 samples (samples 1–29) of cassia bark and the barks of related species were collected from China, Korea, and Vietnam (Table 1). Samples 1–6 were from the Guangxi province, China. Samples 15 and 29 were from Vietnam and Korea, respectively. A total of 15 samples (samples 30–44, representing four types of commodities) were purchased from local drug stores or companies in Hong Kong (Table 2).

Table 1. Collected Crude Materials of Cortex Cinnamomi and Its Related Species

sample	latin name	Chinese name	part	collected location
1	<i>C. cassia</i> Presl	Rou-Gui	bark	Guangxi, China
2	<i>C. cassia</i> Presl	Rou-Gui	bark	Wuzhou, Guangxi, China
3	<i>C. cassia</i> Presl	Rou-Gui	bark	Rongxian, Guangxi, China
4	<i>C. cassia</i> Presl	Rou-Gui	bark	Tengxian, Guangxi, China
5	<i>C. cassia</i> Presl	Rou-Gui	bark	Fangcheng, Guangxi, China
6	<i>C. cassia</i> Presl	Rou-Gui	bark	Pingnan, Guangxi, China
7	<i>C. cassia</i> Presl	Rou-Gui	bark	Mengzi, Yunnan, China
8	<i>C. cassia</i> Presl	Rou-Gui	bark	Yunnan, China
9	<i>C. cassia</i> Presl	Rou-Gui	bark	Deqing, Guangdong, China
10	<i>C. cassia</i> Presl	Rou-Gui	bark	Luoding, Guangdong, China
11	<i>C. cassia</i> Presl	Rou-Gui	bark	Fujian, China
12	<i>C. cassia</i> Presl	Rou-Gui	bark	Hainan, China
13	<i>C. cassia</i> Presl	Rou-Gui	bark	Xishuangbanna, Yunnan, China
14	<i>C. cassia</i> Presl var. <i>macrophyllum</i> Chu	Qing-Hua-Gui	bark	Guangxi, China
15	<i>C. cassia</i> Presl var. <i>macrophyllum</i> Chu	Qing-Hua-Gui	bark	Vietnam
16	<i>C. wilsonii</i> Gamble	Chuan-Gui	bark	Sichuan, China
17	<i>C. wilsonii</i> Gamble	Chuan-Gui	bark	Ya'an, Sichuan, China
18	<i>C. wilsonii</i> Gamble	Chuan-Gui	bark	Jiangyou, Sichuan, China
19	<i>C. wilsonii</i> Gamble	Chuan-Gui	bark	Jiangyou, Sichuan, China
20	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	bark	Longnan, Guangxi, China
21	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	twig	Chongyi, Jiangxi, China
22	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	bark	Chongyi, Jiangxi, China
23	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	bark	Lichuan, Jiangxi, China
24	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	bark	Jinggangshan, Jiangxi, China
25	<i>C. japonicum</i> Sieb	Tian-Zhu-Gui	bark	Zhejiang, China
26	<i>C. burmannii</i> (Nees) Blume	Yin-Xiang	twig	Xishuangbanna, Yunnan, China
27	<i>C. mairei</i> Levl	Yin-Ye-Gui	bark	Guanxian, Sichuan, China
28	<i>C. mairei</i> Levl	Yin-Ye-Gui	bark	Anxian, Sichuan, China
29	<i>C. loureirii</i> Nees	Liu-Shi-Gui	bark	Korea

Table 2. Market Samples of Cassia Bark and Related Materials Purchased in Hong Kong

sample	commercial name or grade	original species	dealer ID	price (HK\$) (HK\$/kg)	usage
30	debarked cortex	<i>C. cassia</i> Presl	A	7368.4	drug
31	debarked cortex	<i>C. cassia</i> Presl	A	7368.4	drug
32	debarked cortex	<i>C. cassia</i> Presl	B	1052.6	drug
33	debarked cortex	<i>C. cassia</i> Presl	C	26 000	drug
34	Qi-Bian-Gui	<i>C. cassia</i> Presl	D	6498.8	drug
35	bark	<i>C. cassia</i> Presl	D	232.1	spice
36	bark	<i>C. cassia</i> Presl	E	78.9	drug
37	bark	<i>C. cassia</i> Presl	B	263.2	drug
38	bark	<i>C. cassia</i> Presl	F	131.6	spice
39	bark	<i>C. cassia</i> Presl	G	376.2	drug
40	twig	<i>C. cassia</i> Presl	E	68.2	drug
41	twig	<i>C. cassia</i> Presl	B	131.6	drug
42	bark	<i>C. cassia</i> Presl	J	83.3	spice
43	bark	<i>C. cassia</i> Presl	K	166.7	spice
44	bark	<i>C. cassia</i> Presl	L	166.7	spice

Quantitative Analyses. In comparison to the use of other solvents including 50% MeOH, acetone, chloroform, and hexane and steam distillation, the amount of the selected four markers in the MeOH extract was the highest. The contents of cinnamaldehyde, cinnamic acid, cinnamyl alcohol, and coumarin in the 44 samples listed in Tables 1 and 2 were determined by HPLC. The HPLC chromatograms were generated at wavelengths of 280 nm (Figure 1A) and 250 nm (Figure 1B). The peaks of coumarin, cinnamic acid, cinnamaldehyde, and cinnamyl alcohol were identified based on the corresponding retention time at 7.88, 11.80, 15.77, and 10.10 min, respectively.

The results showed that the contents of cinnamaldehyde in samples 1–13 ranged from 13.05 to 48.29 mg/g, in samples 14

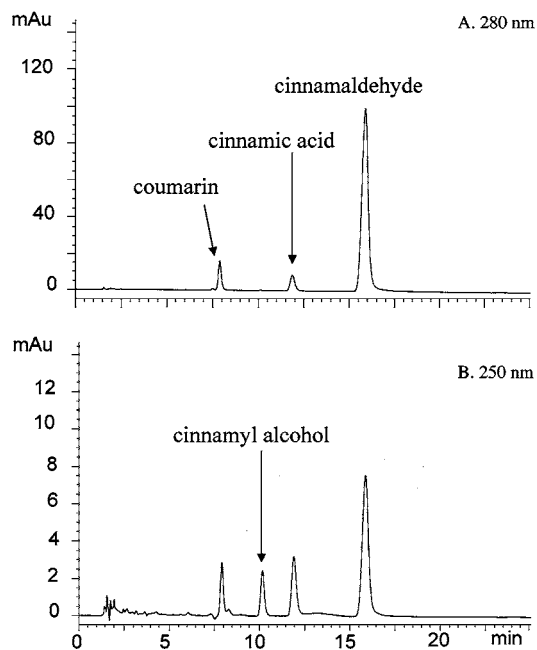


Figure 1. HPLC chromatograms of cassia bark. (A) 280 nm, to determine the contents of coumarin, cinnamic acid, and cinnamaldehyde. (B) 250 nm, to determine the content of cinnamyl alcohol.

and 15, ranged from 51.91 to 56.93 mg/g, and in sample 29, was 12.74 mg/g (Figure 2 and Table 3). The contents of cinnamaldehyde in the bark of other *Cinnamomum* species (samples 16–28) were below 2.00 mg/g.

Samples of four market commodities purchased in Hong Kong were also studied. A large variation in prices was found (Table 2). The cassia twigs (samples 40 and 41) were the cheapest, priced at HK\$ 68.2–131.6/kg, while debarked cortex (samples 30–32) were the most expensive, from HK\$ 1052.6–26 000/kg. Analytical results seemed to suggest there was a direct relationship between price and the contents of cinnamaldehyde. The highest content of cinnamaldehyde was found in sample 30 (debarked cortex), amounting to 93.83 mg/kg. The lowest content of cinnamaldehyde was in sample 40 (twigs). Also, it was shown that the average content of cinnamaldehyde in samples 30–33 (debarked cortex) and sample 34 (Qi-Bian-Gui) was 65.35 mg/kg. Cinnamic acid, cinnamyl alcohol, and coumarin are the minor components of cassia bark (Figure 3).

Chemical Profile. From the HPLC chromatograms on 13 samples of the bark of *C. cassia* (samples 1–12), five chemical

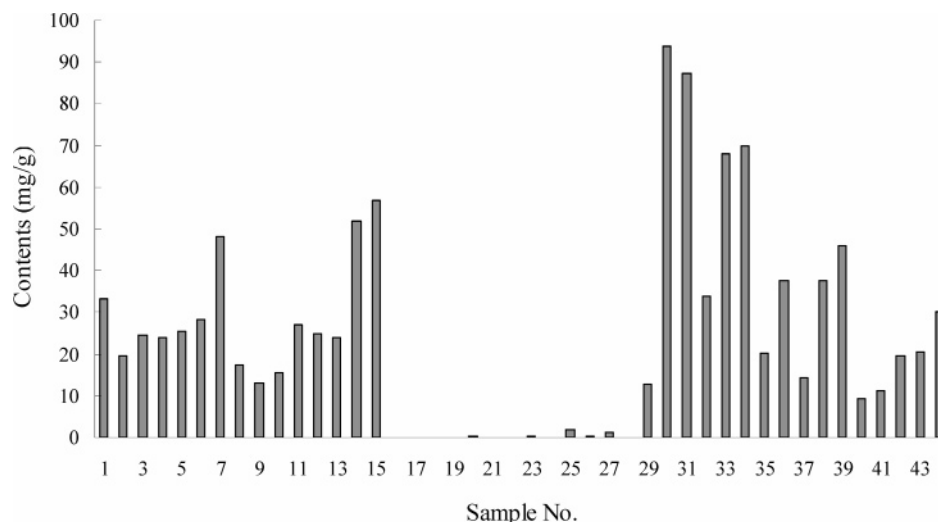


Figure 2. Contents (mg/g) of cinnamaldehyde in the 44 samples listed in Table 3.

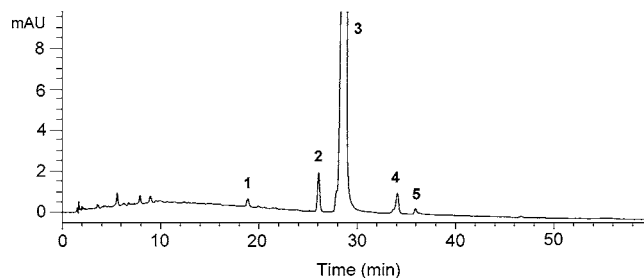


Figure 3. HPLC profile of five components in cassia bark. (1) Coumarin, (2) cinnamic acid, (3) cinnamaldehyde, (4) unknown, and (5) eugenol.

components were selected as characteristic peaks for the fingerprint (Figure 3). Four peaks were identified as coumarin (peak 1), cinnamic acid (peak 2), cinnamaldehyde (peak 3), and eugenol (peak 5), respectively, by comparing the corresponding retention times and the UV spectra of the standards. One undetermined component (peak 4) was also included in the fingerprint. The average relative retention times of these peaks versus cinnamaldehyde (1.00, peak 3) were 0.66 (peak 1), 0.92 (peak 2), 1.20 (peak 4), and 1.27 (peak 5), and their average relative areas ($\times 10E-3$) are 1.00 (peak 3), 6.60 (peak 1), 12.26 (peak 2), 14.11 (peak 4), and 2.04 (peak 5), respectively. Previously, a group did not find eugenol in *C. cassia*, but two groups did observe this chemical (7, 17, 24). Our finding supports the existence of eugenol in *C. cassia*.

On the basis of the established chromatographic profiles, the analytical results of seven selected samples were compared with that of the fingerprint of cassia bark. It was obvious that the bark of *C. cassia* var. *macrophyllum* (sample 14) and debarked cortex (sample 30) were very similar to the given reference (Table 4 and Figure 4), in which all five common peaks were detected but the relative area of coumarin in the chromatogram of debarked cortex was 6.8 times higher than the reference. Furthermore, three peaks 1, 2, and 4 were absent or just barely detectable in the samples of *C. wilsoni*, *C. burmanii*, and *C. mairei* (samples 17, 26, and 28). Peak 2 of *C. japonicum* (sample 23) was missing, and the relative areas of peaks 1, 4, and 5 were significantly different from the fingerprint of cassia bark (Table 4). Peak 5 in the bark of *C. loureirii* (sample 29) was absent. These results revealed that the bark from *C. cassia* could be distinguished from other *Cinnamomum* species by the HPLC fingerprint.

In this study, four main chemical components in 44 samples of cassia bark and related species were determined by the

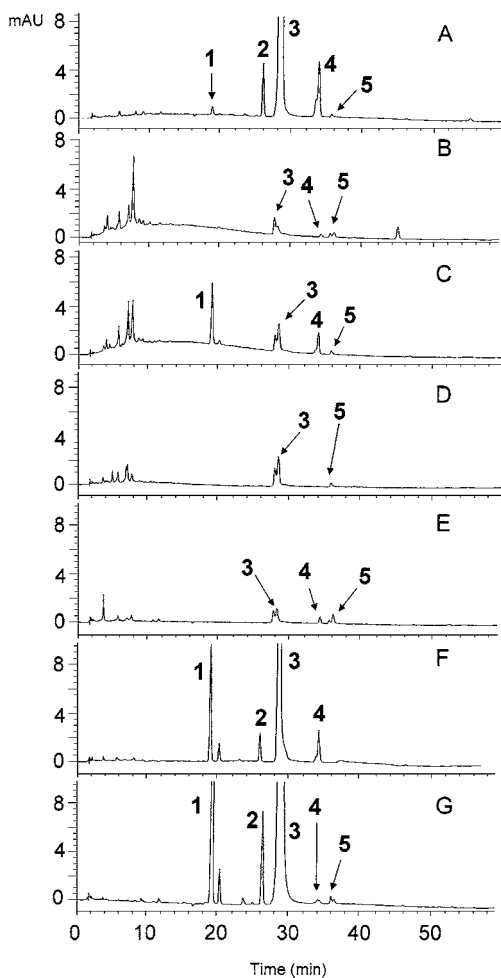


Figure 4. HPLC chromatograms of related *Cinnamomum* species (A) *C. cassia* var. *macrophyllum* (sample 14), (B) *C. wilsoni* (sample 17), (C) *C. japonicum* (sample 23), (D) *C. burmanii* (sample 26), (E) *C. mairei* (sample 28), (F) *C. loureirii* (sample 29), and (G) debarcked cortex (sample 30). (1) Coumarin, (2) cinnamic acid, (3) cinnamaldehyde, (4) unknown, and (5) eugenol.

established HPLC method (Table 3). With reference to the just released Chinese pharmacopoeia (2005 edition), the content of cinnamaldehyde in qualified cortex cinnamomi should reach at least 10.00 mg/g. Table 3 shows the contents of cinnamaldehyde in all 44 samples of cassia bark. The content of cinnamaldehyde in the bark of *C. cassia* was more than 10.00 mg/g. However, the other related *Cinnamomum* species, *C. wilsonii*, *C. japonicum*, *C. burmannii*, and *C. mairei*, would have to be regarded as counterfeits because their contents of cinnamaldehyde were less than 1.00 mg/g.

The price of cortex cinnamomum is traditionally based on the size, thickness, and smell of the bark. In our analysis of the market commodities, it was apparent that the price was directly proportional to the content of cinnamaldehyde (Table 3). The content of cinnamaldehyde may thus be taken as an important index for prizing and quality assessment. The contents of cinnamaldehyde in samples 16–28 were lower than 10.00 mg/g; thus, it was possible to differentiate *C. cassia* from counterfeits by comparing the contents of cinnamaldehyde.

Five major peaks were selected as the fingerprints of cassia bark and related species (Figure 4). Three peaks (peak 1, 2, and 4) were absent or barely detectable in the chromatogram of *C. wilsoni*, *C. burmanii*, and *C. mairei*, while peak 2 was absent in *C. japonicum*. The areas of peaks 1 and 4 in *C.*

Table 3. Contents of Cinnamaldehyde, Cinnamic Acid, Cinnamyl Alcohol, and Coumarin in Cassia Bark and Related Materials (mg/g)

sample	cinnamaldehyde	cinnamic acid	cinnamyl alcohol	coumarin	sample	cinnamaldehyde	cinnamic acid	cinnamyl alcohol	coumarin
1	33.28	0.45	0.85	0.04	23	0.33	0.01		1.26
2	19.46	0.22	0.07	0.19	24	0.08			0.22
3	24.53	0.28	0.01	0.11	25	1.99	0.02		0.03
4	24.02	0.25	0.03	0.85	26	0.38		0.02	
5	25.47	0.19	0.05		27	1.29	0.04		0.20
6	28.36	0.22	0.18	0.13	28	0.14			
7	48.29	0.39	0.06	0.12	29	12.74	0.25	0.16	2.33
8	17.28	0.19	0.02	0.11	30	93.83	0.84	0.69	9.17
9	13.05	0.18	0.01	0.63	31	87.14	0.83	0.69	9.13
10	15.56	0.16	0.05	0.15	32	33.83	1.91	0.44	5.44
11	27.04	0.29	0.07	0.85	33	67.98	1.38	0.15	12.18
12	24.92	0.30	0.13	0.37	34	69.76	0.87	0.15	4.27
13	23.89	0.47	1.77	0.46	35	20.10	0.35	0.10	0.88
14	51.91	0.51	0.23	0.18	36	37.52	0.39	0.10	0.21
15	56.93	0.82	0.06	0.87	37	14.36	0.31	0.27	0.41
16	0.07	0.01			38	37.47	0.83	0.07	1.83
17	0.08				39	45.87	0.73	0.42	6.13
18	0.05				40	9.44	0.40	0.18	0.41
19	0.13				41	11.12	0.40	0.63	0.35
20	0.16			0.33	42	19.50	0.33	0.09	0.43
21	0.08			0.21	43	20.39	0.76	0.31	0.13
22	0.06			0.01	44	29.99	0.55	0.14	1.88

Table 4. Relative Areas of Common Peaks for Related *Cinnamomum* Species

sample	relative areas				
	peak 1 ($\times 10^{-3}$)	peak 2 ($\times 10^{-3}$)	peak 3 (S)	peak 4 ($\times 10^{-3}$)	peak 5 ($\times 10^{-3}$)
14	1.650	11.059	1.000	14.509	0.819
17			1.000		381.976
23	1629.690		1.000	694.864	124.248
26			1.000		125.238
28			1.000		165.128
29	73.766	18.172	1.000	20.885	
30	44.954	9.902	1.000	0.915	0.780

japonicum (sample 23) were 247 and 49 times higher than those of *C. cassia* (Table 4 and Figure 4). The barks of *C. loureirii* could be distinguished from the bark of *C. cassia* by the absence of peak 5. In comparison to the fingerprint of cassia bark, the bark of *C. cassia* var. *macrophyllum* and debarcked cortex was identified as having the best quality. The results showed that the quantity of cinnamaldehyde and HPLC fingerprint analysis along with corresponding chemical components may be used for rapid and reliable authentication and quality assessment of cassia bark.

NOTE ADDED AFTER ASAP PUBLICATION

The original posting of March 11, 2005, contained an error in the Chemical Profile paragraph. This has been corrected as of March 15, 2005.

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