Tissue-specific chemical profiling and quantitative analysis of bioactive components of *Cinnamomum cassia* by combining laser-microdissection with UPLC-Q/TOF–MS

Wenwen Zhou¹,², Zhitao Liang², Ping Li¹, Zhongzhen Zhao²* and Jun Chen¹*

**Abstract**

**Background:** Cinnamomi Cortex, the dried stem bark of *Cinnamomum cassia* Presl (Rougui in Chinese) has been widely used in traditional Chinese medicine, cooking and perfumery for thousands of years. Traditionally, the Cinnamomi Cortex of thick size is considered to be of good quality; however, there is no scientific data to support this point. Considering that essential oils are the main bioactive components, Cinnamomi Cortex of greater variety and amount essential oils is thought to be of better quality. In this study, laser microdissection coupled with ultra-high performance liquid chromatography-quadrupole/time-of-flight-mass spectrometry (UPLC-Q/TOF–MS) was applied to profile the essential oils in different tissues of Cinnamomi Cortex and to determine if there is a correlation between the essential oil content and the stem bark thickness.

**Results:** We report the tissue-specific metabolic profiles of different grades of Cinnamomi Cortex. Nineteen chemical components were unequivocally or tentatively identified in the chromatogram of the test samples. The results indicate that the bioactive components, the essential oils, were mainly present in the phloem.

**Conclusion:** Phloem thickness is the key character for evaluating the quality of Cinnamomi Cortex. Our results can be of great importance in improving the cultivation, harvesting, and processing of Cinnamomi Cortex, as well as enhancing its effects in clinical applications.

**Keywords:** Essential oils, *Cinnamomum cassia* Presl, LMD, UPLC-Q/TOF–MS
such as Zhong tong (cylindric as sample RGgxdzxt), Ban gui (plate-like as sample RGgxpnbg), and Guan gui (scroll-like or groove shape as sample RGgxpnngg). In clinical applications, they are typically used without discrimination, but is there a clinical difference? Comparing the chemical composition of different grades will enable us to determine the difference between grades and will help us evaluate whether these differences are significant in terms of applications. Modern laboratory studies have focused on HPLC-based fingerprint chromatography and determination of characteristic components [8–10]. However, evaluating the quality of Cinnamomi Cortex by modern instruments is time-consuming and inconvenient. Traditionally, the Cinnamomi Cortex of thick size is thought to be of good quality; but there is no scientific evidence to support this point. In the present study, various samples of Cinnamomi Cortex of different grades were collected for tissue-specific chemical analysis combining laser micro-dissected system (LMD) with ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-Q/TOF–MS). Through this study, the relationship between microscopic features and active components can be established; this relationship will enable people to evaluate pharmaceutical quality of Cinnamomi Cortex by appearance. The research also provides helpful information that can guide the cultivating, collecting and processing of Cinnamomi Cortex for maximum quality in applications.

Experiment section

Plant materials

The plant materials were collected from six major cultivation areas. Table 1 shows the details including sources and morphological descriptions for each sample. Figure 1 shows the characteristic appearance of a sample. All the plant materials were identified by Prof. Zhongzhen Zhao, School of Chinese Medicine, Hong Kong Baptist University. The voucher specimens are deposited in the Bank of China (Hong Kong) Chinese Medicines Centre of Hong Kong Baptist University.

Chemicals and reagents

Chemical standards including coumarin, cinnamyl alcohol, cinnamic acid, cinnamaldehyde and 2-methoxy-cinnamaldehyde were purchased from Shanghai Tauto Biotech Company (Shanghai, China). The purity of each standard was over 98%. Acetonitrile and methanol of HPLC grade were from E. Merck (Darmstadt, Germany), and formic acid of HPLC grade was from Tedia (Fairfield, USA). Water was purified using a Milli-Q water system (Millipore; Bedford, MA, USA).

Materials and instruments

Leica Laser microdissection 7000 system (Leica, Bensheim, Germany), Agilent 6540 ultra-performance liquid chromatography quadrupole time of flight spectrometer equipped with a mass hunter workstation software (Agilent version B.06.00 series, Agilent Technologies, USA), Cryotome (Thermo Shandon As620 Cryotome, Cheshire, UK), Ultrasonic instrument (CREST 1875HTAG Ultrasonic Processor, CREST, Trenton, NJ), Centrifuge (Centrifuge 5417R, Eppendorf, Hamburg, Germany), Electronic balance (Mettler Toledo MT5 style), Nonfluorescent polyethylene terephthalate (PET) microscope steel frame slide (76 × 26 mm, 1.4 μm, Leica Microsystems, Bensheim, Germany), Centrifuge tube (500 μL, 1.5 mL, Leica), HPLC grade vial (1.5 mL, Grace, Hong Kong), glass insert with plastic bottom spring (400 μL, Grace, Hong Kong), Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters, USA), C18 pre-column (2.1 × 5 mm, 1.7 μm, Waters, USA).

Sample solution preparations

The dried medicinal materials were firstly softened by infiltrating with water-soaked paper. The softened Cinnamomi Cortex was cut into small sections, fixed by cryogen, and then frozen on a −20 °C cryobar. Serial slices of 40 μm in thickness were cut at −10 °C. Each cross-section of tissue was mounted directly to a non-fluorescent polyethylene terephthalate. The slide was exposed under a Leica LMD 7000 microscopic system. Microdissection was conducted by a DPSS laser beam at 349 nm wavelength, aperture of 30, speed of 3, power of 50 μJ and pulse frequency of 1695 Hz under a Leica LMD system at 6.3 × magnification. Four different target tissues, approximately 1 × 10⁴ μm² per each, were individually separated. The microdissected tissues fell into caps of 500 μL micro centrifuge tubes by gravity. Lastly, the separated tissue part in each cap was transferred to the bottom of the tube by centrifuging for 10 min (12,000 rpm, 17 °C). 100 μL methanol was added into each micro centrifuge tube. The tube was sonicated for 60 min and then centrifuged again for 10 min (12,000 rpm, 17 °C). 90 μL of the supernatant was transferred into a glass insert with plastic bottom spring in a 1.5 mL brown HPLC grade vial and stored at 4 °C before analysis.

Standard solution preparation

Each standard compound was accurately weighed by an analytical balance and dissolved in methanol to produce mixed stock solution with concentrations at 103.05 μg/mL of coumarin, 12.32 μg/mL of cinnamyl alcohol, 132.7 μg/mL of cinnamic acid, 106.94 μg/mL of...
cinnamaldehyde, 157.6 μg/mL of 2-methoxycinnamaldehyde. A series of mixed standard solutions was prepared by dilution with methanol.

Method of UPLC-Q/TOF–MS

The UPLC-Q/TOF–MS analysis was conducted at room temperature (20 °C). The mobile phase consisted of 0.1% formic acid–water (A) and 0.1% formic acid-acetonitrile (B). The gradient program was optimized as follows: 0–8 min, 5–35%; 8–21 min, 35–65%; 21–27 min, 65–100%; 27–31 min, 100%; 31–31.1 min, 100–5%; 31.1–35 min, 5%. The injection volume was 3 μL for each sample. The flow rate was set at 0.4 mL/min. The mass spectra was acquired in positive mode with mass to charge ratio (m/z) ranging from 100 to 1700. The operation parameters of the mass spectrometer were set as follows: dry gas temperature, 300 °C; dry gas (N2) flow rate, 8.0 L/min; nebulizer pressure, 40 psi; capillary voltage, 3500 V; nozzle voltage, 500 V; and fragmentor voltage, 120 V. The energies for collision-induced dissociation (CID) for fragmentation were set at 20 and 35 eV.

Method validation

Linearity, limits of detection (LODs), limits of quantification (LOQs), repeatability, stability, intra-day precision and inter-day precision were assessed. A series of diluted

| Sample no. | Locality       | Grade    | Morphological description                                                                 | Mean thickness (mm) | Proportions of each tissue (%) |
|------------|----------------|----------|------------------------------------------------------------------------------------------|---------------------|---------------------------------|
|            |                |          | Surface                                                                                   | Cross-section       | CK  | C  | PE | PH |
| Dgyujeaj   | Wen'an, Vietnam| Grade A  | Externally greyish-white, slightly rough, showing greyish-green streak, internally reddish-brown | Pericycle banded    | 3.7 | 6  | 13 | 5  | 76 |
| Dgyuebj    | Wen'an, Vietnam| Grade B  | Both externally and internally reddish-brown, slightly even                                 | Pericycle banded    | 3.0 | –  | 20 | 14 | 66 |
| Dgyuecj    | Wen'an, Vietnam| Grade C  | Externally greyish-brown, slightly rough, showing greyish-white streak, internally reddish-brown | Pericycle banded    | 3.1 | 6  | 17 | 11 | 66 |
| Dgxdjcy    | Guangxi, China | Not specific | Externally greyish-brown, slightly rough, internally pale brown                           | Pericycle banded    | 3.1 | 7  | 24 | 28 | 41 |
| Dgxpjcy    | Guangxi, China | Not specific | Externally brown, slightly rough, internally brownish-red                               | Pericycle banded    | 2.4 | 4  | 20 | 11 | 65 |
| Dgqdcqcy   | Guangdong, China| Not specific | Externally greyish-brown, relatively rough, internally pale brownish                 | Pericycle banded    | 4.1 | 5  | 27 | 28 | 40 |
| Dgxdzt     | Guangxi, China | Zhong tong | Externally greyish-brown, slightly rough, internally dark brown                      | Pericycle banded    | 3.7 | 4  | 29 | 25 | 42 |
| Dgxpznt    | Guangxi, China | Zhong tong | Externally pale brown, slightly rough, internally dark brown                           | Pericycle scattered | 5.9 | 5  | 32 | 38 | 25 |
| Dgqdzqt    | Guangdong, China| Zhong tong | Externally greyish-brown, slightly rough, internally brownish-red                   | Pericycle scattered | 4.7 | 10 | 17 | 24 | 49 |
| Dgynaj     | Yunnan, China  | Grade A  | Externally greyish-brown, relatively rough, showing greyish-white or greyish-green streak, internally reddish-brown | Pericycle banded | 4.1 | 7  | 16 | 10 | 67 |
| Dgynbj     | Yunnan, China  | Grade B  | Externally greyish-brown, relatively rough, showing greyish-white or greyish-green streak, internally reddish-brown | Pericycle banded | 4.3 | 2  | 21 | 38 | 39 |
| Dgunjc     | Yunnan, China  | Grade C  | Externally greyish-brown, relatively rough, showing greyish-white or greyish-green streak, internally reddish-brown | Pericycle scattered | 3.8 | 5  | 24 | 26 | 45 |
| Dgxpngb    | Guangxi, China | Ban gui  | Externally dark brown, slightly rough, internally brownish-red                        | Pericycle banded    | 6.0 | 6  | 31 | 21 | 42 |
| Dgxdxbg    | Guangxi, China | Ban gui  | Externally greyish-brown, slightly rough, internally dark brownish-red              | Pericycle scattered | 2.4 | 5  | 31 | 29 | 35 |
| Dgw        | Laos         | Not specific | Externally greyish-brown, slightly rough, internally dark brown                    | Pericycle banded    | 3.0 | 6  | 27 | 34 | 33 |
| Dgxpnnig   | Guangxi, China | Guan gui | Externally dark brown, slightly rough, internally pale brown | Pericycle banded    | 3.6 | 4  | 55 | 16 | 25 |
mixed standard solutions was analyzed subsequently from low to high concentration for linearity, LODs and LOQs. The phloem of RGyueaj was selected for validating the method’s repeatability and stability. Repeatability was evaluated by six replicated analyses of the phloem at the similar locations in six tissue slices. Stability was tested on one sample solution at 0, 12, 24, 36, 48 h. Intra-day precision was performed by analyzing five replications of the mixed standard solution in 1 day while inter-day precision was examined by analyzing three replications of the solution in three consecutive days.

**Results and discussion**

**Microscopic examination and dissection by LMD**

As shown under the normal light and fluorescence mode (Fig. 2), the transverse section of Cinnamomi Cortex could be divided into four portions: cork (CK), cortex (C), pericycle (PE) and phloem (PH). Cork consists of several layers of cells and emits bluish-grey fluorescence. Cortex has a scattering of stone cells. Dark brown fluorescence was emitted from cortex to phloem, while a bright blue color was emitted from the pericycle. Pericycle was arranged in an interrupted ring. Phloem was broad with rays 1–2 rows of cells wide. Since different tissues possessed various features and could be distinguished under fluorescence mode, each separated tissue was dissected at the size of about 1,000,000 μm² by LMD.
Tissue-specific chemical profiling

Tissue-specific chemical profiles were obtained as base peak chromatograms by UPLC-Q/TOF–MS (representative chromatograms are showed in Fig. 3). A total of 19 peaks were unequivocally or tentatively identified in the chromatogram of the medicinal material sample RGYuncj by comparing their retention times, m/z of molecular ions and/or fragment ions with standards or reported references [2, 11–16]. Five peaks were positively identified. Peaks 11, 13, 14, 15 and 16 were unambiguously identified as coumarin ([M+H]⁺), cinnamic acid ([M+H]⁺), cinnamaldehyde ([M+H]⁺), cinnamyl alcohol ([M+H]⁺) and 2-methoxycinnamaldehyde ([M+H]⁺), respectively. 13 peaks were tentatively identified by comparison of their molecular ions of [M+H]⁺ or [M+Na]⁺ from literature reports. The detailed results are shown in Table 2.

As seen from Table 3, peak 10 couldn’t be detected in any tissue of any sample. It can be assumed that the content of peak 10 is below LOD in herbal tissues. The totality of chemicals in cortex (5–12 peaks) and phloem (5–10 peaks) was slightly greater than those in cork (4–8 peaks) and pericycle (5–8 peaks). Peaks 11, 13, 14, 15, 16, namely coumarin, cinnamic acid, cinnamaldehyde, cinnamyl alcohol and 2-methoxycinnamaldehyde, could be detected in almost every tissue. Distinctly, the areas of these peaks were larger than those of other chemicals. Therefore, further quantitative analysis of them was carried out.

Quantification of essential oils in various tissues

The results of method validation are presented in Table 4. The regression equation for each compound was calculated in the form of \( y = ax + b \), where \( y \) and \( x \) were peak area and amount of compound injected, respectively. Each calibration curve possessed good linearity with correlation coefficients \( r^2 \) ≥ 0.9953 within the selected range. The LODs and LOQs were determined at signal-to-noise (S/N) ratios of 3 and 10, respectively. The repeatability ranged from 5.34 to 27.56%. The RSD value of stability was less than 11.66%, indicating that the stability of current method in this study was acceptable. The above assay results indicate that this developed method is reproducible, precise and sensitive enough for tissue-specific determination of five analytes in Cinnamomi Cortex.

The results of quantitative analysis (Additional file 1: Table S1 and Fig. 4) demonstrated that the content of cinnamaldehyde was much higher than other chemicals. Cinnamaldehyde was concentrated in phloem except for sample RGlyw, where it was most abundant in the pericycle. 2-methoxycinnamaldehyde showed the same pattern...
| Peak no. | Identification | t₀ (min) | Molecular formula | Measured mass (m/z) | Theoretical mass (m/z) | Mass accuracy (ppm) | Ion type | MS/MS (m/z) |
|---------|----------------|----------|------------------|--------------------|-----------------------|---------------------|----------|------------|
| 1       | Fructose<sup>a</sup> | 0.71     | C₆H₁₂O₆         | 203.0522           | 203.0532              | −4.92              | [M + Na]<sup>+</sup> | 185[M+Na-H₂O]<sup>+</sup>, 157[M+Na-CH₂O]<sup>+</sup>, 136[M+HCHO]<sup>+</sup> |
| 2       | Sucrose<sup>a</sup> | 0.71     | C₁₂H₂₂O₁₁       | 365.1048           | 365.1060              | −3.29              | [M + Na]<sup>+</sup> | 351[M+Na-CH₂O]<sup>+</sup>, 203[M+Na-C₆H₅O]<sup>+</sup> |
| 3       | (+)-Catechin<sup>a</sup> | 3.33     | C₁₅H₁₄O₈        | 291.0856           | 291.0863              | −2.40              | [M + H]<sup>+</sup> | 185[M+H-C₆H₅O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |
| 4       | Procyanidin B1 or B2<sup>a</sup> | 3.34     | C₉H₁₄O₁₂        | 579.1484           | 579.1497              | −2.24              | [M + H]<sup>+</sup> | 409[M+H-C₅H₈O]<sup>+</sup>, 309[M+H-C₆H₅O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |
| 5       | B-type procyanidin tetramer<sup>a</sup> | 3.92     | C₁₅H₁₈O₁₈       | 867.2116           | 867.2131              | −1.73              | [M + H]<sup>+</sup> | 579[M+H-C₅H₈O]<sup>+</sup>, 439[M+H-C₆H₅O]<sup>+</sup>, 377[M+H-C₅H₈O]<sup>+</sup>, 344[M+H-C₆H₅O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |
| 6       | Procyanidin B1 or B2<sup>a</sup> | 3.92     | C₁₅H₁₄O₁₂       | 579.1487           | 579.1497              | −1.73              | [M + H]<sup>+</sup> | 439[M+H-C₅H₈O]<sup>+</sup>, 344[M+H-C₆H₅O]<sup>+</sup>, 289[M+H-C₅H₈O]<sup>+</sup> |
| 7       | B-type procyanidin tetramer<sup>a</sup> | 4.10     | C₁₂₀H₁₀₀O₁₅₆    | 1155.2741          | 1155.2765             | −2.08              | [M + H]<sup>+</sup> | 867[M+H-C₅H₈O]<sup>+</sup>, 579[M+H-C₆H₅O]<sup>+</sup>, 483[M+H-C₅H₈O]<sup>+</sup>, 351[M+H-C₆H₅O]<sup>+</sup>, 171[M+H-C₅H₈O]<sup>+</sup> |
| 8       | Cinneol<sup>a</sup> | 4.67     | C₉H₁₄O₇         | 407.2037           | 407.2046              | −2.21              | [M + Na]<sup>+</sup> | 349[M+Na-C₅H₈O]<sup>+</sup>, 331[M+H-C₆H₅O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |
| 9       | Cinnamic acid<sup>b</sup> | 5.20     | C₁₅H₁₄O₉         | 537.2297           | 537.2312              | −2.79              | [M + Na]<sup>+</sup> | 303[M+Na-C₅H₈O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |
| 10      | Guaiacol<sup>a</sup> | 6.23     | C₈H₈O₂          | 147.0438           | 147.0422              | 10.88              | [M + Na]<sup>+</sup> | 118[M+Na-CHO]<sup>+</sup>, 103[M+Na-C₆H₄O]<sup>+</sup> |
| 11      | Coumarin<sup>b</sup> | 6.23     | C₇H₆O₂          | 147.0438           | 147.0440              | −1.36              | [M + H]<sup>+</sup> | 103[M+H-CO]<sup>+</sup>, 91[M+H-C₆H₄O]<sup>+</sup>, 77[M+H-C₆H₄O]<sup>+</sup>, 65[M+H-C₅H₈O]<sup>+</sup> |
| 12      | 2-Hydroxycinnamaldehyde<sup>a</sup> | 6.40     | C₆H₈O₂          | 149.0592           | 149.0597              | −3.35              | [M + H]<sup>+</sup> | 131[M+H-C₆H₄O]<sup>+</sup>, 121[M+H-CO]<sup>+</sup>, 103[M+H-C₅H₈O]<sup>+</sup> |
| 13      | Cinnamic acid<sup>b</sup> | 7.79     | C₈H₆O₄          | 149.0595           | 149.0597              | −1.34              | [M + H]<sup>+</sup> | 131[M+H-CO]<sup>+</sup>, 123[M+H-C₇H₆O]<sup>+</sup>, 103[M+H-C₆H₄O]<sup>+</sup> |
| 14      | (E)-Cinnamaldehyde<sup>b</sup> | 8.28     | C₇H₈O          | 133.0647           | 133.0648              | −0.75              | [M + H]<sup>+</sup> | 115[M+H-CO]<sup>+</sup>, 105[M+H-C₆H₄O]<sup>+</sup>, 103[M+H-C₅H₈O]<sup>+</sup> |
| 15      | Cinnamyl alcohol<sup>b</sup> | 9.39     | C₁₀H₁₄O        | 135.0802           | 135.0804              | −1.48              | [M + H]<sup>+</sup> | 117[M+H-C₆H₄O]<sup>+</sup>, 91[M+H-C₅H₈O]<sup>+</sup>, 55[M+H-C₅H₈O]<sup>+</sup> |
| 16      | 2-Methoxycinnamaldehyde<sup>ab</sup> | 9.39     | C₁₀H₁₄O₂        | 163.0750           | 163.0754              | −2.45              | [M + H]<sup>+</sup> | 145[M+H-C₅H₈O]<sup>+</sup>, 135[M+H-CO]<sup>+</sup>, 115[M+H-C₅H₈O]<sup>+</sup>, 107[M+H-C₅H₈O]<sup>+</sup>, 91[M+H-C₅H₈O]<sup>+</sup>, 79[M+H-C₆H₄O]<sup>+</sup>, 57[M+H-C₆H₄O]<sup>+</sup>, 55[M+H-C₅H₈O]<sup>+</sup> |
| 17      | Unknown | 13.00    | C₁₁H₁₈O₂       | 237.1829           | 237.1849              | −8.43              | [M + H]<sup>+</sup> | 71[M+H-C₆H₄O]<sup>+</sup>, 81[M+H-C₅H₈O]<sup>+</sup>, 89[M+H-C₆H₄O]<sup>+</sup> |
| 18      | Dehydro-sesquiterpenoid oxide<sup>a</sup> | 16.56    | C₁₁H₂₂O        | 219.1741           | 219.1743              | −0.91              | [M + H]<sup>+</sup> | 150[M+H-C₅H₈O]<sup>+</sup>, 135[M+H-C₆H₄O]<sup>+</sup>, 121[M+H-C₅H₈O]<sup>+</sup> |
| 19      | Dehydro-sesquiterpenoid oxide<sup>a</sup> | 18.54    | C₁₁H₂₂        | 203.1791           | 203.1794              | −1.48              | [M + H]<sup>+</sup> | 185[M+Na-C₅H₈]<sup>+</sup>, 150[M+H-C₆H₄O]<sup>+</sup>, 136[M+H-C₅H₈O]<sup>+</sup>, 123[M+H-C₅H₈O]<sup>+</sup> |

<sup>a</sup> Identified by previous literature reports  
<sup>b</sup> Identified by standards
of occurrence as cinnamaldehyde. Cinnamic acid was enriched in pericycle of sample RGgxpxjcy and in cork of samples RGgxpnzt and RGlw as well as in phloem of other samples. For all samples, phloem contained the highest amount of coumarin. Cinnamyl alcohol showed the highest content in phloem of one sample, in pericycle of six samples and in cork of others; thus, for this component, the pattern of distribution was difficult to determine. The irregularity may be due to its low content and/or its tendency to esterify easily.

Conclusions
In the present study, an approach using LMD combined with UPLC-Q/TOF–MS was established to map the distribution of essential oils in tissues of various specifications of Cinnamomi Cortex. It is the first report with

| Sample no. | Tissues/peak no. (T: totality) | CK | T | C | T | PE | T | PH | T |
|------------|--------------------------------|----|---|---|---|----|---|----|---|
| RGyueaj    | 1, 2, 11, 12, 13, 14, 15, 16 | 8  | 1, 2, 5, 9, 11, 12, 13, 14, 15, 16 | 11 | 1, 2, 11, 13, 14, 15, 16 | 7  | 1, 2, 11, 13, 14, 15, 16 | 7 |
| RGyuebj    | 1, 2, 11, 12, 13, 14, 15, 16 | 8  | 1, 2, 3, 4, 6, 9, 11, 13, 14, 16 | 10 | 1, 2, 4, 11, 14, 16 | 6  | 1, 2, 4, 9, 11, 13, 14, 15, 16 | 9 |
| RGyuec    | 1, 2, 11, 13, 14, 15, 16 | 7  | 1, 2, 4, 5, 7, 9, 11, 13, 14, 15, 16 | 11 | 1, 2, 11, 13, 14, 15, 16 | 7  | 1, 2, 11, 12, 13, 14, 15, 16 | 8 |
| RGgxpxjcy | 11, 14, 16 | 4  | 2, 4, 8, 11, 13, 14 | 6  | 2, 8, 9, 11, 13, 14, 15, 16 | 8  | 2, 8, 11, 13, 14, 16 | 6 |
| RGgxpnjcy | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5 |
| RGgddqjcy | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5 |
| RGgxdxzjcy | 2, 11, 13, 14, 15, 16 | 6  | 2, 4, 6, 8, 11, 13, 14, 15, 16 | 9  | 2, 11, 13, 14, 15, 16 | 6  | 2, 11, 13, 14, 15, 16 | 6 |
| RGgxpxznz | 2, 11, 13, 14, 15, 16 | 6  | 2, 5, 6, 8, 11, 13, 14, 15, 16 | 10 | 2, 11, 13, 14, 15, 16 | 6  | 2, 11, 13, 14, 15, 16 | 6 |
| RGgddqjzt | 1, 11, 13, 14, 15, 16 | 6  | 1, 4, 5, 7, 8, 11, 13, 14, 15, 16 | 10 | 1, 2, 11, 13, 14, 15, 16 | 7  | 1, 2, 4, 5, 8, 11, 13, 14, 15, 16 | 10 |
| RGyunaj   | 11, 13, 14, 15, 16 | 5  | 4, 5, 7, 11, 12, 13, 14, 15, 16 | 9  | 11, 13, 14, 15, 16 | 5  | 2, 11, 13, 14, 15, 16 | 6 |
| RGyunbj   | 1, 4, 11, 13, 14, 15, 16 | 6  | 1, 4, 5, 11, 13, 14, 15, 16 | 8  | 1, 2, 11, 13, 14, 15, 16 | 7  | 1, 2, 11, 12, 13, 14, 15, 16 | 8 |
| RGyuncj   | 1, 11, 13, 14, 15, 16 | 6  | 2, 4, 5, 7, 9, 11, 13, 14, 15, 16 | 12 | 1, 11, 12, 13, 14, 15, 16 | 7  | 1, 11, 12, 13, 14, 15, 16 | 8 |
| RGgxpbng  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5 |
| RGgxdbng  | 11, 12, 13, 14, 15, 16 | 6  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5  | 11, 13, 14, 15, 16 | 5 |
| RGlw | 2, 8, 11, 12, 13, 14, 15, 16 | 8  | 2, 8, 9, 11, 12, 13, 14, 15, 16 | 9  | 2, 11, 12, 13, 14, 15, 16 | 7  | 1, 2, 8, 11, 13, 14, 15, 16 | 8 |
| RGgxpnbg  | 11, 13, 14, 15, 16 | 5  | 2, 4, 6, 8, 11, 13, 14, 15, 16 | 7  | 2, 11, 13, 14, 15, 16 | 6  | 2, 11, 13, 14, 15, 16 | 6 |

| Table 4 Method validation results |
|----------------------------------|---|---|---|---|---|---|---|---|
| Analyte                          | Calibration curve | Linear range (ng/mL) | r² | LODs (ng/mL) | LOQs (ng/mL) | Repeatability (n = 6, RSD, %) | Stability (n = 5, RSD, %) | Precision RSD (%) |
| Coumarin                         | y = 905852x – 26008 | 51.525–1305 | 0.9981 | 19.1 | 56.1 | 17.43 | 5.99 | 3.17 | 2.81 |
| Cinnamalcohol                    | y = 1486.4x – 350.23 | 267.6–11339 | 0.9970 | 29.0 | 147.3 | 27.56 | 2.03 | 6.13 | 32.66 |
| Cinnamic acid                    | y = 66690x – 2038 | 663.5–1327 | 0.9982 | 159.3 | 334.2 | 5.34 | 7.34 | 4.31 | 5.27 |
| Cinnamaldehyde                   | y = 539.3x + 833.7 | 2615.6–111058 | 0.9996 | 513.2 | 1053.0 | 1037.0 | 3.40 | 2.45 | 30.60 |
| 2-Methoxycinnamaldehyde          | y = 1*10^6x – 5380.3 | 39.4–394 | 0.9953 | 9.3 | 52.7 | 9.26 | 11.66 | 23.97 | 28.40 |
respect to tissue-specific metabolites in the cortex of an herb. This histochemical study identified Cinnamomi Cortex phloem as the tissue richest in essential oils. Thus, it would be logical to deduce that Cinnamomi Cortex with thick phloem is of better quality as it contains more active constituents. In fact, this is consistent with the traditional processing method of removing the outer bark. Our analytical method provides references for evaluating the quality and classifying the grades of Cinnamomi Cortex by thickness of phloem. Further studies can be conducted to explore the factors affecting phloem thickness. Therefore, this research can be of great importance in the cultivation, harvesting, processing and clinical application of Cinnamomi Cortex.

Additional file

Additional file 1: Table s1. Contents of essential oils in various tissues of the samples.

Authors’ contributions

WZ and ZL initiated and all authors designed the study. WZ carried out the histochemical experiment and drafted the manuscript. PL and ZZ provided technical support. All authors contributed to the data analysis and to finalizing the manuscript. ZZ has made his intellectual contributions in authenticating the materials. JC contributed her intellectual content for revising the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

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References

1. Zhang GZ, Zhang SN, Meng QH, Wang XD (2009) GC-MS analysis on chemical components of Cortex Cinnamomi and Guipi. Chin J Pharm Anal 29:1256–1259
2. Chen PY, Yu JW, Lu FL, Lin MC, Cheng HF (2016) Differentiating parts of Cinnamomum cassia using LC-qTOF-MS in conjunction with principal components analysis. Biomed Chromatogr 30:1449–1457
3. Yuan PF, Shang MY, Cai SQ (2012) Study on fingerprints of chemical constituents of Cinnamomi ramulus and Cinnamomi cortex. Chin J Chin Mater Med 37:2917–2921
4. Mathew S, Abraham TE (2006) Studies on the antioxidant activities of cinnamon (Cinnamomum verum) bark extracts, through various in vitro models. Food Chem 94:520–528
5. Subash Babu P, Prabuseenivasan S, Ignacimuthu S (2007) Cinnamaldehyde: a potential antidiabetic agent. Phymed 14:15–22
6. An FL, Zhang Z, Xiang CK, Kang LF (2009) Component analysis of essential oils from Cinnamomum and their inhibition action against platelet aggregation. Chin Pharm 18:25–27
7. Giordani R, Regli P, Kaloustian J, Portugal H (2006) Potentiation of antifungal activity of amphotericin B by essential oil from Cinnamomum cassia. Phytother Res 20:58–61
8. Ding Y, Wu EQ, Liang C, Chen JB, Tran MN, Hong CH, Jang Y, Park KL, Bae K, Kim YH, Kang JS (2011) Discrimination of cinnamon bark and cinnamon twig samples sourced from various countries using HPLC-based fingerprint analysis. Food Chem 127:755–760
9. He ZD, Qiao CF, Han QB, Cheng CL, Xu HX, Jiang RW, But PH, Shaw PC (2005) Authentication and quantitative analysis on the chemical profile of cassia bark (Cortex Cinnamomi) by high-pressure liquid chromatography. J Agric Food Chem 53:2424–2428
10. Huang YT, Pan T, Wen J, Tang XY, Sun YS, Chi L, Peng P, Shi RB (2015) Quality representation and correlation analysis of the characteristic spectrum of Rougui based on drug system. J Beijing Univ Tradit Chin Med 38:344–350
11. Wei L, Song YL, Guo XY, Tu PF, Jiang Y (2014) Habitat differentiation and degradation characterization of Cinnamomi Cortex by 1H NMR spectroscopy coupled with multivariate statistical analysis. Food Res Int 67:155–162
12. Shan B, Cai YZ, Brooks JD, Corke H (2007) Antibacterial properties and major bioactive components of Cinnamon Stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. J Agric Food Chem 55:5484–5490
13. Chen P, Sun JH, Ford P (2014) Differentiation of the four major species of cinnamons (C. burmannii, C. verum, C. cassia, and C. loureiroi) using a flow injection mass spectrometric (FIMS) fingerprinting method. J Agric Food Chem 62:2516–2521
14. Liao SG, Yuan T, Zhang C, Yang SP, Wu Y, Yue JM (2009) Cinnacassides A-E, five geranylphenylacetate glycosides from Cinnamomum cassia. Tetrahedron 65:883–887
15. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW, Graves DJ (2004) isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem 52:65–70
16. Avula B, Smilliea TJ, Wang YH, Zweigenbaum J, Khan IA (2014) Authentication of true cinnamon (Cinnamomi Cortex verum) utilising direct analysis in real time (DART)-QToF-MS. Food Addit Contam Part 32:1–8

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