

1 **【Original Papers】**

2 **Comparison of Various Commercially Available Cinnamon Barks using NMR**

3 **Metabolomics and the Quantification of Coumarin by Quantitative NMR Methods**

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5 Ryuichiro Suzuki,^{*}¹ Yuki Kasuya,¹ Aiko Sano,¹ Junki Tomita,¹ Takuro Maruyama,²

6 Masashi Kitamura¹

7

8 ¹ Laboratory of Natural Products & Phytochemistry, Department of Pharmaceutical

9 Sciences, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado

10 City, Saitama, 350-0295, Japan

11 ² Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of

12 Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki City, Kanagawa,

1 210-9501, Japan

2 *Corresponding author

3 Tel and Fax: +81 49 271 8089

4 E-mail: ryu_suzu@josai.ac.jp

5 ORCID: 0000-0002-3492-1671

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1 **Abstract**

2 Cinnamon bark is an important spice worldwide. In this study, the chemical diversity of
3 various commercially available cinnamon barks that differed in their production areas
4 and utility applications (culinary spice or medicines) were investigated by the use of ^1H
5 NMR metabolomics. Our results indicated that principle component analysis (PCA) and
6 hierarchical cluster analysis (HCA) of the ^1H NMR spectra of the cinnamon bark
7 methanolic extracts including deduction of their species by nucleotide sequence analysis
8 enabled differentiation of the cinnamon barks according to their species, production
9 areas and utility applications. The constituents of Vietnam cinnamon were found to
10 differ significantly from the other samples investigated based on PCA score plots and
11 HCA constellation dendrograms. Coumarin was found to be a key compound for the
12 discrimination of Vietnamese cinnamon by multivariate analysis of the ^1H NMR

1 spectral data and direct comparison of the ^1H NMR spectra. In addition, coumarin was
2 quantified using quantitative NMR methods. As the results, coumarin was contained in
3 Vietnamese cinnamon at higher level compared to other cinnamons. This study
4 indicated that ^1H NMR metabolomics could deduce spices, utility, and producing area of
5 commercially available cinnamon barks. Furthermore, combining quantitative ^1H NMR
6 methods with ^1H NMR metabolomics enable quantification of coumarin in cinnamon
7 bark on single measurement.

8

9 *Keywords:* cinnamon bark, metabolomics, coumarin, quantitative NMR

10

11

1 **Introduction**

2 The genus *Cinnamomum* (family: Lauraceae) consists of over 250 varieties, four of
3 which are widely used as a culinary spice, namely Chinese cinnamon (*C. cassia*),
4 Indonesian cinnamon (*C. burmanii*), Vietnamese cinnamon (*C. loureiroi*), and Ceylon
5 cinnamon (*C. zeylanicum*).[1] The cinnamon obtained from these sources is used for the
6 flavoring of various foods and beverages, including boiled beef, pickles, chutneys, and
7 ketchup.[2] The true cinnamon (*C. verum*; often designated with the synonym *C.*
8 *zeylanicum*), otherwise known as Laurus cinnamon or Ceylon cinnamon, is produced as
9 a single or double compound from quill bark, whereas Chinese cinnamon, which
10 originates from *C. cassia*, is mainly produced as flat, curved, channeled simple
11 corticated bark pieces. Currently, *C. verum* is extensively used in pharmaceutical
12 preparations, seasonings, and cosmetics. Compared to Chinese cinnamon, true

1 cinnamon is a highly valued spice that is regarded as being superior compared to the
2 readily available and cheaper cassia, since the latter has a weaker aroma and a more
3 astringent taste due to the high tannin content present in the cork tissue compared to that
4 found in *C. verum* bark.[3]

5 Indeed, the quality of cinnamon bark as a natural spice depends on various diverse
6 factors, such as its species and the environmental conditions during growth.

7 The volatile oil composition of *C. verum* bark is mostly dominated by
8 phenylpropanoids, with (*E*)-cinnamaldehyde being present as the major compound that
9 accounts for the distinct taste and scent. Other chemical components of *C. verum* bark
10 oil include coumarin, cinnamoyl alcohol, cinnamic acid, and eugenol, as determined by
11 steam distillation. In contrast, the major volatiles present in *C. cassi* oil were found to
12 be (*E*)-cinnamaldehyde, coumarin, and cinnamoyl acetate.[3] In addition, NMR

1 metabolomics allowed two cinnamon species to be distinguished, namely Ceylon
2 cinnamon (*C. verum*) and Chinese cinnamon (*C. cassia*), and the marker components for
3 each species were revealed (i.e., eugenol for *C. verum* and fatty acids for *C. cassia*).[3]
4 In another report, differentiation of the cinnamon barks of the four major species (i.e., *C.*
5 *burmannii*, *C. verum*, *C. aromaticum*, and *C. loureiroi*) was achieved using a mass
6 spectrometric method.[4] Cinnamon is also of interest due to its medicinal properties,
7 having been employed in the treatment of gastric disturbances, blood circulation
8 disorders, and inflammatory disease. Other medicinal effects of cinnamon include
9 antifungal, antioxidant, antiallergy, and anti-diabetic properties.[5,6]

10 In the context of this study, metabolite fingerprinting approaches based on nuclear
11 magnetic resonance (NMR) spectroscopy can generate valuable metabolite signatures
12 for complex plant extracts. The advantage of ^1H NMR spectroscopy over other

1 metabolomics techniques is that the signal intensity depends only on the molar
2 concentration of each compound in the solution, thereby enabling direct comparisons of
3 the concentrations of all compounds present in the sample. In our previous reports, we
4 demonstrated that an NMR-based metabolomics approach could distinguish between
5 different *Glycyrrhiza* species (i.e., *G. glabra*, *G. uralensis*, and *G. inflata*),[7] and
6 identify the habitats of *Sophora flavescens* (i.e., Japan or China).[8,9] Furthermore, an
7 NMR metabolomics approach was employed to screen the antimicrobial activity of
8 yucca extracts used as a food additive.[10]

9 Proton-specific quantitative NMR (qNMR) has also attracted attention for the
10 analyses of natural products[11] due to its advantages over quantitative HPLC methods.
11 Such qNMR methods do not require the use of reference compounds for establishing
12 calibration curves, nor is sample pre-purification required, and the simultaneous

1 determination of multiple constituents in foods and natural medicines can be
2 achieved.[12,13] Importantly, qNMR methods are compatible with NMR metabolomics,
3 and these can be conducted through sequential studies.

4 Thus, we herein report the investigation of twelve commercially available cinnamon
5 samples used for culinary spices or medicines through the use of ^1H NMR spectroscopy
6 coupled to multivariate statistical analysis.

7 Firstly, we deduce their species on the basis of nucleotide sequence analysis of the
8 internal transcribed spacer 2 (ITS2) region of rDNA, which is useful for Cinnamon
9 species identification.[14] Based on the experimental data, we evaluate a comprehensive
10 profile of cinnamons based on their species, geographical origins and utility
11 applications. Absolute quantifications of the key compound present in the twelve
12 cinnamons are also determined using quantitative NMR methods.

1 **Materials and methods**

2 **Reagents and Chemicals**

3 DSS-*d*₆ (sodium 3-(trimethylsilyl)-1-propane-1,1,2,2,3,3-*d*₆-sulfonate) (Code no.
4 044-31671, Lot., purity: 92.4±0.5%) was used as a reference material for quantitative
5 NMR measurements, and was supplied by FUJIFILM Wako Pure Chemical Corporation
6 (Chuo-ku, Osaka, Japan). Coumarin was also purchased by FUJIFILM Wako Pure
7 Chemical Corporation (Chuo-ku, Osaka, Japan). Dimethylsulfoxide-*d*₆ (DMSO-*d*₆) was
8 obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA).

9

10 **Cinnamon Samples**

11 Details of cinnamon samples are shown in Table 1. The commercial cinnamon samples
12 employed herein were obtained from UCHIDAWAKANYAKU Ltd. (UW, Arakawa-ku,

1 Tokyo, Japan), S&B FOODS INC. (SB, Chuo-ku, Tokyo, Japan), SPICE SHOP
2 RASALHANUT (SR, Tokorowaza, Saitama, Japan), GABAN Co., Ltd. (GB, Chuo-ku,
3 Tokyo, Japan), Sakurai Foods Co., Ltd. (SF, Minokamo, Gifu, Japan), Takasago
4 Yakugyo Co., Ltd. (TY, Abeno, Osaka, Japan), Eidaiyakugyo Co., Ltd. (EY, Osaka,
5 Osaka, Japan), and Tochimoto Tenkaido Co., Ltd. (TT, Osaka, Osaka, Japan). All
6 samples were obtained as the dried entire bark tissue. Specimens are maintained at the
7 Laboratory of Natural Products and Phytochemistry, Department of Pharmaceutical
8 Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University.

9

10 **DNA sequence analysis**

11 Approximately 20 mg of each sample was crushed with an MM400 mixer mil (Retsch,
12 Haan, Germany). Genomic DNA was extracted from the powdered sample using a

1 DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The partial sequence of ITS2 region
2 of nuclear rDNA was amplified by 2 times polymerase chain reaction (PCR) using the
3 obtained genomic DNA as a template.

4 The pair of primers for amplification of the ITS2 regions were given as follows:
5 5'-GCA TCG ATG AAG AAC GTA GCG-3' and 5'-TTT TCC TCC GCT TAT TGA
6 TAT GC-3' for 1st round PCR and 5'-GCC CGA GGC CAC TC-3' and 5'-GGC CAC
7 GCC TGA CCT G-3' for 2nd round PCR. PCR was performed on a DNA engine
8 PTC-200 (Bio-Rad, Hercules, CA) using BIOTAQ HS DNA polymerase (Bioline,
9 London, UK) and Ampdirect plus (Shimadzu, Kyoto, Japan) with the following
10 program-95°C for 10 min; followed by 50 cycles of 95°C for 30 s, 50°C (1st round) or
11 60°C (2nd round) for 30 s and 72°C for 45 s; 72°C for 7 min. After purification of the
12 reaction mixture using a MinElute PCR Purification Kit (Qiagen), the amplicon was

1 directly sequenced by Fasmac co. ltd. using a BigDye Terminator v3.1 Cycle
2 Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI 3130xl or 3730
3 Genetic Analyzer (Applied Biosystems). The DNA sequences were aligned using the
4 Clustal W program. The botanical origin of each sample was deduced by the direct
5 comparison of the sequences from authentic samples and/or the corresponding sequence
6 on the International Nucleotide Sequence Database (DDBJ/EMBL/GenBank; INSD).

7

8 **Sample Preparation and Extraction**

9 Each dried ground cinnamon powder (10 g) was extracted with methanol under reflux
10 for 1 h, and this was repeated three times for each sample. The methanolic extracts were
11 then evaporated to give dried residues. These residues were dissolved in DMSO- d_6 at a
12 concentration of 10 mg/mL for NMR measurements.

1

2 **Instrumentation**

3 One- and two-dimensional NMR spectra were recorded at 400 MHz on an Agilent
4 400MR-vnmrs 400 spectrometer (Agilent Technologies, Inc., Santa Clara, CA). For the
5 quantitative NMR measurements, a Varian NMR System 700 was used with a
6 $^1\text{H}\{^{13}\text{C}/^{15}\text{N}\}$ 5 mm Triple Resonance ^{13}C Enhanced Cold Probe, VT, 700NB.

7

8 **^1H NMR Analysis for Metabolomics**

9 The ^1H NMR spectra were recorded at 20°C on a 400 MHz Agilent 400MR-vnmrs
10 400 spectrometer. Each spectrum consisted of 65,536 complex data points and had a
11 spectral width of 6410.3 Hz. The spectra were obtained over 16 scans with a repetition
12 time of 5.0 s and relaxation delay of 1.50 s per scan. The detection pulse flip angle was

1 set at 45°.

2

3 **¹H NMR Spectrometer and Parameters for Quantitative NMR Measurements**

4 The ¹H NMR spectra were recorded on a Varian NMR System 700 spectrometer (700

5 MHz) under the following conditions: number of scans: 32, dummy scans: 2, relaxation

6 delay time: 62.35 s, data acquisition time: 2.330 s, spectral width: -5.0 to 15.0 ppm

7 (14044.9 Hz), 90° pulse length: 9.35 μs, sample temperature: 293 K, spinning: OFF.

8 Chemical shifts are given in ppm values relative to the proton signal of DSS-*d*₆ (0.00

9 ppm). Integral values of the proton signals were obtained after Fourier transform of the

10 free-induction decay (FID) data. Phase correction, integration range setting, and

11 baseline correction were performed manually. Data analysis were performed using Delta

12 (ver. 5.3.0, JEOL, Tachikawa, Tokyo, Japan).

1 Integration of the signals was set to ranges that included both ^{13}C satellite signals.
2 Baseline correction was performed using a linear algorithm at both ends of each
3 integration range.

4

5 **Data Analysis: Principal Component Analysis (PCA) and Hierarchical Cluster**
6 **Analysis (HCA)**

7 Each NMR spectrum was divided into 350 regions (0.04 ppm wide) in the region
8 between 0.00 and 14.00 ppm. Each segment of the spectral region was then integrated.
9 Any integrated regions from 1.12 to 1.24 ppm, 2.40 to 2.52 ppm, and 3.24 to 3.36 ppm
10 that contained solvent and water signals were eliminated from the data table, such that
11 the total data were reduced to 341 regions. The remaining integral values for each
12 spectrum were normalized over 100 total summed integrals to compensate for any

1 differences in concentration among the cinnamon extracts. Spectral processing was
2 performed using ALICE2 for Metabolome version 5.0 (JEOL). Multivariate analyses
3 such as principal component analysis (PCA) and hierarchical cluster analysis (HCA)
4 were conducted using JMP Pro 13 (SAS Institute Inc., Cary, NC).

5

6 **Quantitative NMR (qNMR) Measurements of Coumarin**

7 The methanol extract of the cinnamon sample was accurately weighed to ~10 mg
8 using a micro-balance, while DSS- d_6 was weighed accurately to ~1.0 mg. The cinnamon
9 sample and DSS- d_6 were then placed in a vial and dissolved in DMSO- d_6 (1000 μ L),
10 and an aliquot (600 μ L) of the solution was transferred into an NMR tube. Three
11 experimental samples for qNMR were prepared for each cinnamon bark sample.

12 For the quantification of coumarin using NMR spectroscopy, the peak area of the

1 selected proton signal belonging to coumarin (δ_{H} 8.02, H-4), and the peak area of the
2 internal standard (DDS- d_6) were integrated manually. The following equation was
3 applied for the calculations.

$$4 \quad Pa = \frac{S_a}{S_s} \times \frac{N_s}{N_a} \times \frac{M_a}{M_s} \times \frac{m_s}{m_a} \times Ps;$$

5 where Pa and Ps are the molar ratios of coumarin and DDS- d_6 , respectively; S_a and S_s
6 are the signal integrals at δ_{H} 8.02 (coumarin) and δ_{H} 0.00 (DDS- d_6); N_a and N_s are the
7 number of protons producing the signals at δ_{H} 8.02 (coumarin, CH \times 1) and δ_{H} 0.00
8 (DDS- d_6 , CH $_3$ \times 3); Ma and Ms are the molecular weights of coumarin and DDS- d_6
9 (146.15 and 224.36, respectively); and m_a and m_s are the sampling weights of coumarin
10 and DDS- d_6 , respectively.

11

12 **Results and Discussion**

1 In the present study, twelve different cinnamon samples, which were divided into three
2 groups according to their production area (i.e., Vietnam, China, or Sri Lanka) were
3 employed, as outlined in Table 1. These samples were also separated into two groups
4 based on their applications, i.e., as a culinary spice or as a medicine, as specified by the
5 Japanese Pharmacopeia (JP-grade).[15] Furthermore, their ITS2 partial sequences were
6 determined by DNA sequence analysis. The obtained sequences were applied to a
7 similarity search using the BLAST (Basic Local Alignment Search Tool) program and
8 the botanical origin was deduced. The results are summarized in Table 1. According to
9 alignments of the sequences, the sequences of *C. cassia* were discriminated from those
10 of *C. verum/dubium*. #5, 6, and 7 used as culinary spice were identified as *C.*
11 *verum/dubium* and others were identified as *C. cassia*. A spice cinnamon #4 was
12 identified to *C. cassia*.

1 All 341 variables in the bucketed regions were equally accounted for in the data sets.

2 The PCA models were depicted as score plots and consisted of two synthetic variables,

3 namely principal component (PC)1 (the greatest data variance) and PC2 (the second

4 greatest data variance, orthogonal to PC1). The PCA score plot clearly indicated two

5 groups, i.e., cinnamon used as a spice and medicinal cinnamon. This result was

6 consistent with that of DNA analysis. #5, 6, and 7 were identified as *C. verum/dubium*

7 and others were identified as *C. cassia*. The spice cinnamon #4, which was identified to

8 *C. cassia* located far from other spices (#5, 6, and 7) on PCA score plot. The medicinal

9 cinnamon samples were further divided into their production regions, and the

10 Vietnamese cinnamon samples were categorized according to the different suppliers

11 (Fig. 1). In addition, an HCA constellation dendrogram of the cinnamon extracts was

12 grouped into different patterns based on the PCA results (Fig. 2). On the HCA

1 constellation dendrogram, the cinnamon extracts were divided into two groups, namely
2 #2, 9, and 12 (produced in Vietnam and JP-grade) and “others,” whereby the “others”
3 group was divided according to the sample utility (i.e., spice or medicine). Since the
4 medicinal cinnamon extracts produced in Vietnam (#2, 9, and 12) were categorized far
5 from the others on the HCA constellation dendrogram, a PCA loading plot was
6 employed to find the most relevant variables. The result indicated that the ^1H NMR
7 signals observed at δ_{H} 6.46 and δ_{H} 8.02 are characteristic for Vietnam cinnamon #9.
8 Furthermore, the signal at δ_{H} 2.90 and δ_{H} 9.62 are characteristic for spice cinnamons #5,
9 6, and 7 and Chinese cinnamon #3, 8, and 11, respectively. (Fig. 3) The ^1H NMR signal
10 at δ_{H} 9.62 could be assigned to aldehyde proton of cinnamaldehyde and/or 2-hydroxy
11 cinnamaldehyde. Furthermore, the proton signal at δ_{H} 3.86 was observed in cinnamon
12 barks used as JP-grade. This signal also could be assigned to methoxy proton of

1 2-methoxyl cinnamaldehyde. The proton signals appeared at 3-4 ppm which were
2 characteristic for culinary spice (#4, 6, and 7) on PCA loading plot could be assigned to
3 sugar protons. On the other hand, the proton signals at δ_H 7.42 and δ_H 7.70 on PCA
4 loading plot were specific for culinary spice #5. The cinnamon bark (#5) located apart
5 from others on PCA score plot. The signal at δ_H 7.70 could be attributed to olefin proton
6 of cinnamic acid. The signals at δ_H 6.46 and δ_H 8.02 specific for Vietnam were clearly
7 observed only in extract of cinnamon cultivated in Vietnam (#9) (Fig. 4). To identify the
8 origin of these two signals, two-dimensional NMR spectroscopy was carried out,
9 including COSY, HSQC, and HMBC measurements. The coupling constants of these
10 signals were both 10.0 Hz, indicating that these signals correlate to one other, and so
11 they were attributed to the olefinic protons in the *cis* form. In addition, in the HMBC
12 spectrum, the signal at δ_H 8.02 correlated to the ester carbonyl carbon atom (δ_C 161).

1 Since a previous literature reported that some cinnamon bark samples contained
2 coumarin,[16] which possesses both olefinic protons and a carbonyl carbon atom, the
3 ¹H NMR spectrum of coumarin was directly compared to that of cinnamon extract # 9.
4 As a result, signals δ_H 6.46 and δ_H 8.02 were assigned to H-3 and H-4, respectively (Fig.
5 4).

6 Coumarin is a natural flavoring substance present in a variety of plants, including
7 cinnamon bark and cherry blossom leaves. Indeed, coumarin is an important flavoring
8 agent in the food and beverage industry, and can be easily detected in
9 cinnamon-flavored foods and food supplements.[17] However, animal experiments have
10 shown that when administrated as a drug, coumarin has the potential to cause liver
11 damage, according to a BfR (Bundesinstitute für Risikobewertung) assessment. More
12 specifically, the BfR derived a tolerable daily intake (TDI) for of 0.1 mg of coumarin

1 per kg of bodyweight.[18] Although quantification of coumarin in natural medicines by
2 HPLC analysis were conducted,[19-21] by ^1H qNMR is limited.[22,23] At least there
3 are no recent study that quantify coumarin in cinnamon bark by ^1H qNMR. Therefore,
4 the quantification of coumarin was conducted using quantitative NMR methods. More
5 specifically, in the ^1H NMR spectrum of twelve cinnamon bark extracts, the resonance
6 at δ_{H} 8.02 assigned to H-4 was clearly observed, and so this signal was selected as the
7 target signal for quantitative analysis. For the purpose of this investigation, DDS- d_6 was
8 used as internal standard for the NMR measurements, whereby the absolute purity of
9 coumarin was determined from the ratio of the integrated signal intensity of the
10 characteristic coumarin signal (δ_{H} 8.02) to that of DDS- d_6 . Thus, the coumarin content
11 (%) of twelve cinnamon bark extracts were determined to be shown in Table 2. All
12 cinnamon barks without cinnamon used as culinary spice (#5, 6, and 7) were identified

1 as *C. cassia* by nucleotide sequence analysis illustrated in Table 1. Thus, the difference
2 of coumarin content of each cinnamon bark was caused by their production area, not
3 their genetic difference. Overall, we provided a metabolomic investigation of cinnamon
4 bark by means of ^1H NMR experiments and multivariate analysis, and the metabolic
5 fingerprints of cinnamon bark used as a spice and as a medicine were found to differ.
6 Moreover, the different metabolic profile of Vietnamese cinnamon bark was attributed
7 to the presence of coumarin at higher levels compared to other cinnamon barks. Since
8 PCA or HCA deals with all detectable ^1H NMR signal on ^1H NMR spectroscopy, it
9 might be not appropriate to focus on one compound. However, it can be inferred that
10 difference of coumarin content in cinnamon bark affect not a little classification on PCA
11 or HCA. Moreover, the coumarin content (%) present in Vietnamese cinnamon bark was
12 successfully quantified by quantitative NMR methods. This study indicated that ^1H

1 NMR metabolomics could deduce spices, utility, and producing area of commercially
2 available cinnamon barks. Furthermore, combining quantitative ^1H NMR methods with
3 ^1H NMR metabolomics enable quantification of coumarin in cinnamon bark by single
4 measurement. The qualitative and quantitative observation of cinnamon bark could be
5 conducted on a same spectroscopy. In addition, all samples have identified by
6 nucleotide sequence analysis. Based on this research results, it is possible to construct
7 discrimination model to discriminate spices, utility, and producing area of commercially
8 available cinnamon bark by increasing the number of samples and utilizing other
9 statistical analysis methods such as SIMCA (Soft Independent Modeling Of Class
10 Analogy). Difference of processing of cinnamon bark also could affect results of PCA
11 and HCA. In addition, distinguishing between Chinese and Vietnamese cinnamons of
12 JP-grade and clarifying of characteristic compounds for each cinnamon are very

1 interesting. We are now planning to confirm this point and intend to report it in a later
2 paper.

3

4 **Conflict of Interest**

5 The authors declare no conflict of interest.

6

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10 NMR (qNMR) methods.

11

12

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- 5

1 Tables

2 Table 1 Commercial cinnamon barks used in this study.

Sample No.	Producing area	Use	Supplier	Botanical origin*
1	Yen Bai, Vietnam	JP	UW	<i>C. cassia</i>
2	Yen Bai, Vietnam	JP	UW	<i>C. cassia</i>
3	Guanxi Zhuang, China	JP	UW	<i>C. cassia</i>
4	unknown, China	SP	SB	<i>C. cassia</i>
5	unknown, Sri Lanka	SP	SR	<i>C. verum/dubium</i>
6	unknown, Sri Lanka	SP	GB	<i>C. verum/dubium</i>
7	Kandy, Sri Lanka	SP	SF	<i>C. verum/dubium</i>
8	Guanxi Zhuang, China	JP	TY	<i>C. cassia</i>
9	Thanh Hoa, Vietnam	JP	TY	<i>C. cassia</i>
10	Thanh Hoa, Vietnam	JP	EY	<i>C. cassia</i>
11	Guanxi Zhuang, China	JP	TT	<i>C. cassia</i>
12	Yen Bai, Vietnam	JP	TT	<i>C. cassia</i>

JP: specified by the Japanese Pharmacopeia (JP)

SP: mainly used as a culinary spice

Abbreviations of suppliers are described in the text

*: identified by nucleotide sequence information in ITS2 of rDNA

1 **Table 2 Coumarin content (%) of commercial cinnamon barks used in this study**
 2 **on qNMR experiments.**

3	Sample No.	Coumarin content (%)	%SD
	1	3.83	1.00
4	2	5.38	0.57
	3	0.58	0.06
5	4	1.55	0.33
	5	0.03	0.01
6	6	0.14	0.02
	7	0.01	0.00
7	8	0.29	0.02
	9	4.06	1.93
8	10	3.53	0.51
	11	0.39	0.02
9	12	2.89	0.06

10 (n = 3)

11

1 **Figure legend**

2 **Fig. 1** PCA score plot. PCA score plot of the cinnamon barks, in which 341 variables
3 were equally accounted for in the datasets. The PCA score plot clearly showed that
4 culinary spices (5, 6, and 7) were considerably different from medicinal cinnamons
5 specified by the Japanese Pharmacopeia (JP-grade).

6

7 **Fig. 2** Constellation dendrogram. Constellation dendrogram obtained using the ^1H NMR
8 spectral data of the cinnamon barks as illustrated by hierarchical cluster analysis (HCA).
9 Cinnamon barks were divided in two groups, Vietnamese cinnamon specified by the
10 Japanese Pharmacopeia (JP-grade) (2, 9, and 12) and others. In the “others” group, the
11 culinary spices of Sri Lanka differed from those of China.

12

- 1 **Fig. 3** Loading plot of PCA. δ_H 6.46 and δ_H 8.02 were specific for Vietnamese
- 2 cinnamon bark.
- 3
- 4 **Fig. 4** ^1H NMR spectra of Sri Lanka, Chinese, Vietnamese cinnamon bark extracts and
- 5 coumarin. Arrows indicate characteristic signals at δ_H 6.46 and δ_H 8.02 for Vietnamese
- 6 cinnamon bark.