Cinnamon – Differentiation of Four Species by Linking Classical Botany to an Automated Chromatographic **Authentication System**

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One of the world's oldest spices, cinnamon is also one of the most popular. Species of the genus Cinnamomum offer a variety of extractable oils with aroma and flavor characteristics of importance to the flavor industry, so differentiating cinnamon samples for culinary-based applications is very important. Cinnamon also has reported health benefits associated with specific phytochemical constituents, but its composition can vary greatly depending on species and source region. A substantial amount of the research reported on cinnamon does not provide thorough documentation of the source and taxonomic identification of the study material, a very common issue with studies of food and medicinal plants. In the interest of providing some clarity to the discussion of the health benefits and culinary attributes of the different cinnamon types in the marketplace, we offer the results of a long-term chemotaxonomic study of cinnamon samples sourced from different regions of the world and link those chemical data to classical taxonomic identification of the source plants. We provide details of the effective use of an automated chemotaxonomic analytical method to differentiate cinnamons from various geographic regions. Also included are chromatographic data for the polyphenolic/procyanidin fractions of each species, as cinnamon type-A procyanidins are often the purported source of biological activity in cinnamon and cinnamon extracts.

innamon has been used and consumed for thousands of years. This spice was a valuable commodity in the Middle East, and traders probably began bringing

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cinnamon to Egypt around 2000-1500 BC. Originally used in embalming and incense, cinnamon's culinary attributes and appeals were discovered later, particularly during the Greek and Roman periods. Today, cinnamon is one of the most popular spices; it is widely used in cereal, bakery, and dessert products and is increasingly found as a flavoring component in main courses and side dishes as well.

Cinnamon's purported health benefits, e.g., antidiabetic (1–5), antimicrobial (1, 2), antioxidant (1, 2), and anti-Alzheimer (6), have heightened the interest in this spice and led to increased sales and a considerable amount of research in the past decade (1, 2). The primary focus to date has centered on the impact of cinnamon on diabetes, its mechanism of action, and the identity of the bioactive constituents (3-5, 7-10). At least four systematic reviews and meta analyses of cinnamon's antidiabetic effects have been published recently (11-13). In addition, there have been some promising discoveries related to cinnamon's potential as a preventative or therapeutic agent for Alzheimer's disease (14–16).

Here, we outline the differences in four commercially important species of cinnamon using classical botany and chemotaxonomic differences in cinnamon species from various geographic regions. We also describe the automated chemotaxonomic chromatographic analytical method based on the SherlockTM Microbial Identification System (MIS; 17) used in their characterization.

Materials and Methods

Taxonomy

(a) Sample collection and identification.—Voucher specimens of the four primary Cinnamomum species in commerce were obtained from suppliers in Sri Lanka, Indonesia, China, and Vietnam and compared with the morphology of authentic vouchers held at the Claude E. Phillips Herbarium located on the Delaware State University campus. Further species confirmation for each sample was obtained through deoxyribonucleic acid analyses at (NSF AuthenTechnologies, Petaluma, CA; data not shown). The four species, their synonyms, common names, and origin information are provided below.

- (1) Cinnamomum verum J. Presl (syn. C. zeylanicum Blume).—(a) Common names.—Ceylon cinnamon, Seychelles cinnamon, and true cinnamon.
- (b) Origin.—In ancient times, true cinnamon was imported from Sri Lanka (Ceylon), where it is native. Today, this species is also cultivated in many countries in Asia and the Seychelles Islands.
- (2) Cinnamomum aromaticum Nees (syn. C. cassia auct.).—
 (a) Common names.—Chinese cassia, cassia lignea, Canton cassia, Kwangtung cassia, Kwangsi cassia, Yunnan cassia, and Hunan cassia.
- (b) Origin.—This species probably originated in South China, and in ancient times, it was imported from China and Myanmar (Burma). Today, it is widely cultivated in tropical or subtropical areas of the Fujian, Guangdong, Guangxi, Guizhou, Hainan and Yunnan Provinces of China; Taiwan; India; Indonesia; Laos; Malaysia; Thailand; and Vietnam.
- (3) Cinnamomum burmannii (Nees & T. Nees) Nees ex Blume.—(a) Common names.—Cassia vera, Indonesian cassia, Padang cassia, Batavia cassia, Batavia cinnamon, Korintji cassia, Korintji cinnamon, Java cassia, and Timor cassia
- (b) Origin.—This species is found in the Fujian, Guangdong, Guangxi, Hainan, and Yunnan Provinces of China; India; Indonesia; Myanmar; Philippines; and Vietnam.
- (4) Cinnamomum loureiroi Nees.—(a) Common names.— Saigon cassia, cassia lignea, Vietnam cassia, Danang cassia, Annam cassia, and Tonkin cassia.
 - (b) Origin.—This species is found in Vietnam.

Chemotaxonomy

- (a) Sample preparation.—Two grams ground Cinnamomum spp. raw material were added to a 1.0 L round-bottom flask (standard taper 24/40 joint) along with 250 mL deionized H₂O. The round-bottom flask was connected to a Liebig condenser (Part No. CORNING 2400-400; Corning, Inc., Corning, NY) with a volatile oil trap (5 mL Clevenger style, outer 24/40 and inner 24/40 standard taper joints). Deionized water (5-6 mL) and 2.00 mL toluene (Fisher Optima grade) were added to the Clevenger trap before the distillation process was started. An externally controlled heating mantle was used to bring the mixture to boiling. Once boiling started, the heat was adjusted to maintain a 1 drop/s flow rate (into the Clevenger trap). The solution was refluxed/distilled for 4 h. At this time, the heat was removed, and the solution allowed to cool. The toluene layer was recovered by aspiration with a Pasteur pipette, transferred to a glass vial and dried with approximately 1 g Na₂SO₄ (American Chemical Society grade, anhydrous). The remaining clear liquid was transferred to a 2 mL GC autosampler vial (12 × 32 mm screw cap with PTFE/Silicone/PTFE septa) for subsequent GC analysis.
- (b) External calibration standard.—Nine straight-chain alkanes (nonane through heptadecane, 500.0 mg each, ReagentPlus ≥99%; Sigma-Aldrich) and 125.0 mg 1-octanol and 2-nonanone (ReagentPlus ≥99%; Sigma-Aldrich) were added to a 100 mL volumetric flask and diluted to volume with hexane (HPLC grade, 98.5%; Fisher Chemical) and thoroughly mixed to produce the intermediate standard (5.00 mg/mL for alkanes 9–17 and 1.250 mg/mL for 1-octanol and 2-nonanone). Next, 4.0 mL intermediate standard was transferred to a 100 mL volumetric flask, diluted to volume with hexane (HPLC grade), and thoroughly mixed to produce

the working standard (0.20 mg/mL for alkanes 9–17 and 0.05 mg/mL for 1-octanol and 2-nonanone). A 2 mL GC auto-sampler vial was filled with the working calibration standard and capped; the system calibration standard was evaluated prior to analyzing samples of cinnamon oil obtained as above.

Gas Chromatography

GC profiling was performed using an Agilent 6890N GC (Agilent Technologies, Wilmington, DE) equipped with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with a 10 µL syringe suitable for liquid injections. The GC was also equipped with a flame-ionization detector (FID). The chromatographic separation was performed using a Zebron ZB-1 (Part No. 7HG-G001-22; Phenomenex) capillary column (30 m × 0.25 mm i.d., 1.00 µm film thickness). A split/splitless injector was used in split mode. Samples (2 µL) were introduced into the injector (temperature 235°C, pressure 78.5 kPa, split ratio 44.6:1, split flow 76.8 mL/min, and total flow 84.9 mL/min) with H₂ as the carrier gas. The oven temperature program was 80°C for 2 min, increasing to 220°C at a rate of 4°C/min with no hold time, and then increasing to 310°C at a rate of 30°C/min and held for 5 min. The FID conditions were as follows: temperature 300°C, H₂ flow 30 mL/min, air flow 264 mL/min, and constant He makeup flow 30 mL/min.

The raw chromatographic data obtained were automatically analyzed using a custom modified Sherlock MIS, software version 6.0, developed and marketed by MIDI, Inc. (Newark, DE). The Sherlock MIS software compares the volatile compound profiles of test samples against profiles of cinnamons of verified taxonomy from different geographical origins.

Data Processing and Analysis Software

Chromatographic peaks were carefully identified using correlated GC-MS analyses and peak tables were created using Sherlock Library Generation software. Subsequent annotation and pattern recognition comparative analyses using the compiled cinnamon libraries were performed with the Sherlock Chromatographic Analysis Software. The Sherlock software requires a calibration standard at the beginning of each new or unique sample type to optimize the chromatographic peak recognition and performance of the software system. If the calibration report is free of errors, the sample analysis continues. If the area count response of the largest peak in a sample (in the range of C_9 – C_{17}) is larger than 10 000 000, the following message appears on the sample report: "Sample too concentrated, dilute and rerun." In those instances, the sample extract is diluted with toluene (Optima grade) and rerun to obtain a concentration consistent with the largest peak in the range of 400 000-10 000 000 area counts. After the samples are analyzed by GC, the Sherlock software automatically names all preselected or relevant peaks in the sample and assigns a similarity index (SI) value to the sample based on a comparison to the chemical fingerprint of well-vetted cinnamon library sample entries.

Procyanidins

(a) Sample and standard preparation.—The analytical method for polyphenols was adapted from that reported for the

analysis of procyanidins in cocoa. (18, 19) Ground cinnamon (0.5 g) was extracted with sonication for 30 min in a solution of 5 mL 0.5% acetic acid, 29.5% water, and 70% acetone (AWA). The extract was filtered and analyzed by HPLC. Catechin hydrate (SKU No. C1251; Sigma-Aldrich) was used as an external reference standard for quantifying the monomer and oligomer fractions at degrees of polymerization (DP)-2-10. A stock solution of 0.025 g catechin hydrate was freshly prepared in a volumetric flask with 25 mL AWA (final concentration of 1.0 mg/mL) immediately prior to use. Working standard solutions, with a concentration range of 0.02 to 0.3 mg/mL, were prepared by diluting appropriate aliquots of the stock solution. Individual fractions and total procyanidins were reported as mg/g, catechin equivalent. Method RSD = 7% (n = 14)

(b) HPLC instrument parameters.—Agilent 1200 HPLC with fluorescence detector. Column: Develosil 100 Diol-5, 250 \times 4.6 mm, 5 µm (Nomura Chemical, provided by Mars, Inc.); mobile phase A: 2:98 glacial HOAc-CH₃CN (v:v); mobile phase B: 2:3:95 glacial HOAc-H₂O-CH₃OH (v:v:v); flow: 1 mL/min.; gradient: 7% solvent B for 3 min, ramp to 38% B over 57 min, ramp to 100% B over 3 min, hold for 7 min, and return to 7% B over 6 min.

Results and Discussion

Commercial cinnamon and cassia are derived from four principal species of the genus Cinnamomum from southern and southeastern Asia. Traditionally, color and sensory properties have been key to distinguishing identity and quality, but this has been rendered less effective by the harvesting of very young bark and blending materials from different sources. However, these species can be distinguished by various morphological characteristics, e.g., shape of calcium oxalate crystals, starch granules, fibers, cork fragments, and pitting of medullary rays (Table 1).

The cinnamon species can also be distinguished by analysis of the percent composition of selected volatile oil components found in their respective oils (see Table 2).

A review of the data in Table 2 led us to consider whether a more robust chemotaxonomic differentiation of the four species was possible. Volatile oil profiles of the cinnamon species did exhibit chromatographic differences believed sufficient to discriminate the various species. What we needed was a consistent, standardized approach to the analysis to eliminate experimental variables and a software system to manage the data. Inspiration came in the form of the Sherlock MIS for the identification of bacteria through their fatty acid methyl ester (FAME) profiles (17).

The Sherlock software, as originally conceived and constructed, uses a directed statistical training method to develop models of the chemical signatures of microorganisms. To compensate for the lack of standardized conditions (when working with botanicals that are grown, harvested, and processed under various conditions), Sherlock was modified to cope with higher variances in chemical composition by using a combination of the following approaches: (1) creating statistical breadth of database coverage by analyzing multiple analytes of botanical reference materials and their variants. (2) Compound summing, i.e., treating a set of compounds as a single feature to compensate for mole-mole conversions in biochemical pathways. (3) Compound zeroing, i.e., eliminating overwhelming compounds that mask the contribution of smaller, critical compounds from statistical significance, as well

Table 1. Morphological characteristics

Characteristic		Species								
	C. verum	C. aromaticum	C. burmannii	C. lourieroi						
Ca oxalate crystals	a oxalate crystals Acicular Acicular		Cubic, rectangular, or tabular	Cubic						
Starch granules, µd	Rarely >10	Usually >10	10–20	10–20						
Fibers, µd	Up to 30	Up to 40	20–30	30–50						
Cork fragments	Occasional	Abundant	Very rare	Occasional						
Medullary rays	Rarely pitted	Rarely pitted	Coarsely pitted	Unknown						

Table 2. Distinguishing chemical constituents^{a,b}

	Species, %								
Compound	C. verum	C. aromaticum	C. burmannii	C. lourieroi					
Linalool	5.0-7.9	0–0.1	0.7–4.0	0–5					
Benzaldehyde	1.0-6.9	3.1-5.7	0-8.7	0.8-12.7					
α-terpineol	1.4–3.8	0-0.2	0.2-8.2	0-1.4					
eta-caryophyllene	8.4-14.8	0.3-4.3	3.3-13.3	0-2.6					
O-methoxycinnamaldehyde	3.4-6.2	3.1–24.4	0–2.1	0-11.5					
Cis-cinnamaldehyde	5.0-6.7	3.2-9.7	0-16.4	3.6-21.9					
Cinnamyl acetate	0-24.0	0.1–3.6	0-8.8	0.6-3.9					
Eugenol	3.5–16.0	0–6.0	0-0.8	0-5.6					

Data reported as % of volatile oil obtained from McCormick analytical GC data.

Trans-cinnamic aldehyde is excluded from the analysis as it comprises approximately 85–90% of the volatile oil for all species and would be the primary contributor to the subsequent PCA.

as removing any contaminants from inclusion in the chemical profile. (4) Eigenvalue control, i.e., allowing a chemometric "buffer" around the domain of acceptable matches to account for inherent sample variability in the chemical composition. (5) Quality factors, i.e., determining which small compounds are not to be considered as part of the botanical model based on standard deviation compared with the mean.

When a sample is analyzed, the profile is compared with the chemical signature database. The SI is a value between 0.000 and 1.000, and calculated based on the variance and covariance of the named compounds with 1.000 being a perfect match to the library entry listed. An SI is a value between 0.000 and 1.000 calculated based on the variance and covariance of the named compounds, with 1.000 being a perfect match to the library entry listed. If the top match is greater than 0.500 with no secondary match within 0.100 of the top match, the sample is determined to match the entry listed as the top match. The use of an external calibration mixture provides the information necessary for automatic calculation of Equivalent Chromatographic LocaleTM (ECL; MIDI, Inc.) values for precision chromatography (Figure 1). Because the external

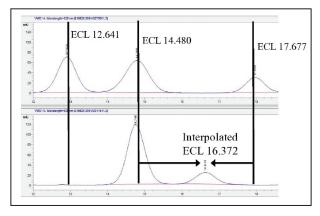


Figure 1. Example of ECL chromatogram calculation.

calibration mixture being reported here uses a sequential series of normal hydrocarbons, the calculated ECL values also correspond to the more familiar Kovat's indexes (20).

The calibration standards create a "picket fence," naming known peaks in the mix, and then allowing for automated interpolation of other peaks. The use of an external calibration minimizes run-to-run and interlaboratory variance and provides quality control parameters (i.e., ensures the instrument is running correctly). An external calibration standard is a proven approach for GC fatty acid analysis of over 100 compounds in the existing MIDI Sherlock platform (17).

To build a robust database, over 100 well-vetted samples of cinnamon bark were obtained from six different geographical regions over the course of 3 decades. A critical factor in this work is that the taxonomy of each collection was verified by botanical taxonomy, and then, the volatile oil of each was produced under identical conditions and evaluated by GC. Table 3 summarizes the collection samples that went into making up the initial database for comparisons with subsequent samples.

The examination of representative chromatograms led to the identification of compounds to be used in a principal component analysis (PCA). Figure 2 illustrates the clustering of species based on a representative PCA.

Table 3. Cinnamon bark samples used in establishing GC profile database

Collection region	Species	No. samples included	No. compounds used		
Sri Lanka	C. verum	5	26		
Seychelles	C. verum	4	29		
Malaysia/Indonesia	C. burmannii	36	34		
Sikiang, China	C. aromaticum	13	38		
Tung Hing, China	C. aromaticum	25	47		
Vietnam	C. lourieroi	22	34		

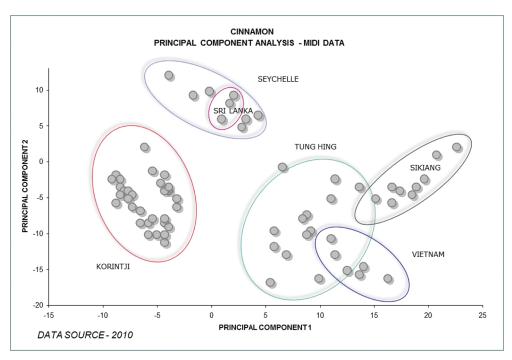


Figure 2. Cinnamon PCA-Sherlock data.

The vetted cinnamon samples gave very reliable volatile oil profiles consistent with each species. Subsequently, this database has been used for comparative analysis of hundreds of cinnamon samples sourced over a period of more than 25 years. The very few cases we have observed in which a volatile oil profile did not match one of the six origins (regarding the four species) were indicative of sample adulteration, or admixing two or more species.

The uniquely identifiable volatile oil profiles of four commercial species of cinnamon are of considerable importance in flavor and fragrance research and food/flavor applications, and those same four species are proving to be of potential commercial significance in the realm of health and wellness. The cinnamon species have differing chromatographic profiles of procyanidins, polyphenols composed of catechin, and/or epi-catechin oligomers, in particular, doubly linked type-A procyanidins (21). These cinnamon procyanidins function as antioxidants and have been shown to exhibit other pharmacological activities, including insulin potentiation and impact on glucose intolerance and diabetes (4, 5, 11–13, 22–24).

The cinnamon procyanidins can be separated and characterized by their DP via HPLC. The patterns and distribution of cinnamon polyphenols appear to be unique for each species/ origin of cinnamon (Figure 3). These chromatograms mimic those published elsewhere on procyanidin separations, most notable in the determination of flavanol and procyanidin content (DP-1–10) of chocolate, cocoa powders and extracts (18, 19).

The relative abundance of procyanidin polymers (DP-1–10) found in the four cinnamon species are listed in Table 4. It is worth noting that abundances for specific polymers (e.g., DP-3 and DP-4) can be highly variable for each species of cinnamon. Specific trimers (e.g., cinnamtannin B-1; 22, 23) and tetramers (e.g., parameritannin A-1; 24) of cinnamon can be present in concentrated forms in cinnamon-derived products (25, 26).

Conclusions

The Sherlock Chromatographic Analysis Software program from MIDI, Inc., originally conceived and designed as a system to identify microorganisms by their FAME profiles, has been successfully adapted to serve as an effective automated botanical authentication system for cinnamon. We anticipate that this same approach will be reported for numerous other botanicals soon. We wish to emphasize that rigorous identification (botanical taxonomy) of any samples used in building a comparative database is essential to the success of such an endeavor.

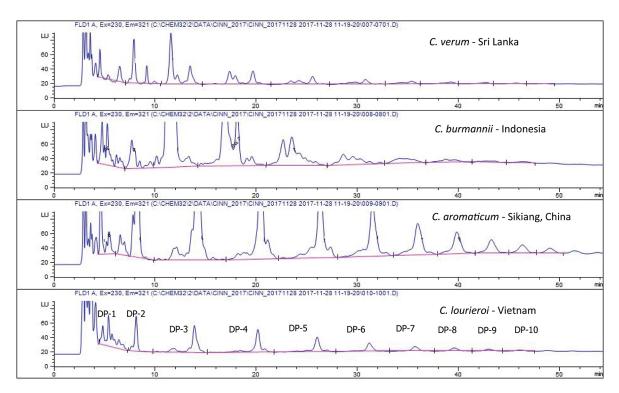


Figure 3. Cinnamon procyanidin HPLC profiles by origin.

Table 4. Cinnamon procyanidin content in samples of known origin

Species (origin)	DP-1	DP-2	DP-3	DP-4	DP-5	DP-6	DP-7	DP-8	DP-9	DP-10	Total DP	DP-3 + DP-4
C. verum (Sri Lanka), mg/g	0.10	0.18	0.30	0.18	0.10	0.06	0.04	0.02	0.01	0.01	1.00	0.48
C. burmannii (Indonesia), mg/g	0.30	0.20	2.33	1.10	0.48	0.22	0.12	0.07	0.03	0.02	4.87	3.43
C. aromaticum (Sikiang, China), mg/g	0.34	1.28	1.11	0.87	0.65	0.47	0.31	0.20	0.13	0.08	5.44	1.98
C. lourieroi (Vietnam), mg/g	0.22	0.11	0.18	0.16	0.12	0.07	0.04	0.03	0.02	0.01	0.96	0.34

The findings reported here suggest that researchers involved in health and wellness or biomedical/pharmacological studies on cinnamon that use cinnamon purchased from the grocery store shelves, health food stores, or other sources with no traceability to product country of origin or species identification other than label claims should confirm the accurate species identification by chemical analysis and avoid the risk of flawed studies from selecting a cinnamon material possibly poorly suited for the intended pharmacological response of interest. In fact, without understanding and proper accounting for the cinnamon species and procyanidin content, past research and corresponding results and conclusions come into question for the validity and subsequent use in comparative or follow-up studies.

We hope that this cinnamon characterization and species clarification model, using robust automated chemotaxonomic methods and based on sound and classical botanical taxonomy, may provide useful guidance to researchers seeking specific cinnamon models for their research on health and wellness attributes or those investigating the culinary, flavor, and fragrance attributes of various cinnamon species.

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