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Analysis of the *trans*-Cinnamic Acid Content in *Cinnamomum* spp. and Commercial Cinnamon Powder Using HPLC

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Abstract

The present study aimed to quantify the content of *trans*-cinnamic acid (CA) in *Cinnamomum japonicum*, *C. loureirii*, and *C. camphora* and the commercial application of CA using high performance liquid chromatography (HPLC). A C18 column was used, with water/methanol as the mobile phase gradient. The highest content of CA was observed in the bark of *C. loureirii* (16.97 mg/g) and cinnamon powder A (47.60 mg/g). The lowest content of CA was observed in leaf and heartwood of *C. japonicum* (0.10 and 0.10 mg/g, respectively) and cinnamon powder C (22.87 mg/g). This result could be utilized as a guideline for the analysis of the commercial applications of *Cinnamomum*.

Keywords

***Cinnamomum* spp., *trans*-Cinnamic Acid, HPLC**

1. Introduction

Cinnamomum spp., which belongs to the Lauraceae family, is an evergreen dicotyledon that includes *C. loureirii*, *C. camphora*, and *C. japonicum* species. Lauraceae includes about 55 genera and more than 2000 species, which are generally grown in warm, tropical, and mild climates [1] [2]. Lauraceae has diverse commercial applications such as food supplements and pesticides. Previous research has demonstrated that the oils of the Himalayan Lauraceae species show anti-oxidant and anti-bacterial properties [3]. Moreover, the essential oils of Lauraceae tree leaves have been screened for cytotoxicity activity [4].

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The term "*Cinnamomum*", meaning "sweet wood", has been derived from the Greek word "*kinnamomon*" [5]. Previous research has revealed that the bark of *C. loureirii* branches contains chemical constituents such as (*E*)-cinnamaldehyde and *α*-copaene. Of the three aforementioned species, *C. japonicum* has the strongest anti-microbial, anti-tyrosinase, and anti-oxidant properties. *C. camphora* also exhibits properties that enable it to be used as natural fumigants and insecticides [6]-[8].

"Cinnamon", the general term for the bark of several *Cinnamomum* spp., has long been used as a food ingredient, because of its sweet and spicy flavor [9]. This spice is used in countries such as India, Madagascar, Sri Lanka, and Vietnam for worldwide distribution [10]. Other research has revealed that cinnamon can prevent contamination by *Salmonella typhimurium* and its use has improved food safety, particularly in dairy applications [11]. Moreover, cinnamon has been shown to substantially improve liver glycogen synthase in case of reduced insulin levels, and the extract from the bark of *C. cassia* has been used to inhibit tumor, angiogenesis, and vascularization [12] [13].

trans-Cinnamic acid (CA), an isomer of cinnamic acid, and its derivative cinnamaldehyde, is found in cinnamon and strawberry. CA has anti-fungal, nematicidal, and anti-microbial properties, and is known for its radio protective effect against skin damage [14]-[18]. Moreover, this substance has anti-oxidant, anti-inflammatory, anti-cancer, and anti-malarial properties [19].

High-performance liquid chromatography (HPLC) has been used in a previous research to analyze cinnamon twigs and bark [20]. In addition, coumarin found in cinnamon has been analyzed by direct inlet probe-atmospheric pressure chemical ionization-mass spectrometry (DIP-APCI-MS) and liquid chromatography-mass spectrometry (LC-MS) [21].

The objective of the present study is to analyze the CA content in *C. loureirii*, of *C. camphora*, and *C. japonicum* as well as in commercially produced cinnamon powder by HPLC.

2. Experimental

2.1. General Experimental Procedure

C. loureirii bark was obtained from Omniherb Co., Ltd. (Yeongcheon, Republic of Korea) and was extracted with methanol (MeOH) under reflux. The MeOH extracts of the leaf of *C. loureirii* and leaf, heartwood, and bark of *C. japonicum* and *C. camphora* were purchased from the Korea Research Institute of Bioscience and Biotechnology (KRIBB; Daejeon, Republic of Korea). Cinnamon powders designated A, B, and C were purchased from a local retailer in Anseong, Republic of Korea.

2.2. Apparatus and Chemicals

Evaporation was conducted with an evaporator system (EYELA rotary vacuum evaporator N1100, Tokyo Radadidai Co. Ltd., Tokyo, Japan) under reflux *in vacuo*. The HPLC analysis was performed by using a Waters Breeze system (Waters Co., Milford, MA, USA) equipped with a Waters 1525 binary HPLC pump and a 2489 system ultraviolet-visible (UV/VIS) detector. HPLC-grade reagents were water containing 0.2% acetic acid and MeOH, and all other reagents were of analytical grade. CA (**Figure 1**) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Preparation of Standards and Samples

The CA was weighed and dissolved in 100% MeOH to obtain a stock standard (1.0 mg/mL). CA aqueous solutions were prepared in concentrations of 100, 10, 1, 0.1, and 0.01 mg/mL for the calibration curve. Then 10.1 g of *C. loureirii* bark was extracted with 250 mL MeOH, and 10 g of each commercial cinnamon powder sample

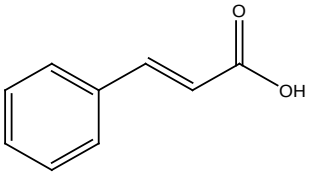

Figure 1. Structure of *trans*-cinnamic acid (CA).

was extracted with 200 mL MeOH under reflux and evaporated *in vacuo*. *Cinnamomum* spp. extracts were dissolved in 1 mL of MeOH and were filtered with a 0.45-μm syringe filter. The dissolved solutions were used for HPLC analysis.

2.4. HPLC Conditions

The quantitative analysis of CA separated by HPLC was performed by using a reverse phase system. Discovery C18 column (250 mm × 4.6 mm, particle size 5 μm) was used, and a mobile phase was a gradient of water and MeOH (20% to 100% over 30 min). An aliquot volume of 10 μl was eluted with a gradient solvent system at a flow rate of 1 mL/min. UV detection was conducted at an absorbance of 280 nm. The flow rate was 1 mL/min.

2.5. Calibration Curve

CA stock solutions (100 - 0.01 μg/mL) were prepared in MeOH. The CA contents of the samples were determined from the corresponding calibration curves. The calibration functions of CA were calculated by using the peak area (Y), concentration (X, μg/mL), and mean values (n = 5) ± standard deviation (SD; **Figure 2**).

3. Results & Discussion

The CA contents of *Cinnamomum* spp. and commercial cinnamon powder are shown in **Table 1**, **Table 2**, and **Figure 3**. The CA concentration in the bark of *C. loureirii* was determined to be 16.97 mg/g of the extract (Omniherb), and that in the leaf of *C. loureirii* was 2.50 mg/g of extract. The CA concentrations in the bark, leaf, and heartwood of *C. camphora*, and *C. japonicum* (KRIBB) were detected to be 0.31, 0.26, and 0.69 mg/g of extract and 0.23, 0.10, and 0.10 mg/g of extract, respectively (**Table 1**). Thus, the CA content of *C. loureirii* was higher than that of the other *Cinnamomum* spp. CA also can be found in cinnamon, virgin olive oil, *Viola betonicifolia*, and strawberries. Previous research has demonstrated that strawberries contain 2.91 to 4.97 μg/g of CA in accordance with maturation stages. Immature green strawberries contain the highest content of 4.97 μg/g [14] [22]. Propolis, also known as bee glue, contains 1% - 1.5% CA [23].

Among the three samples of commercially available cinnamon powder, the content of CA was the highest in cinnamon powder A (47.60 mg/g; **Table 2**). The CA content in all of the powders was higher than that in *Cinnamomum* spp. Generally, cinnamon refers to several *Cinnamomum* spp., and thus percentage values can vary. For examples, a previous study determined that the CA content of the oils of four *Cinnamomum* spp. compositions differed [24]. In addition, commercially produced cinnamon powder includes two types of *Cinnamomum* trees: true cinnamon (*C. verum*) and cassia (*C. aromaticum*). Therefore, the results of CA content analysis can differ [5].

Various studies have been conducted on cinnamon derivatives in commercial products. Ultra performance liquid chromatography (UPLC) has been used to analyze the coumarin content in cinnamon contained in food

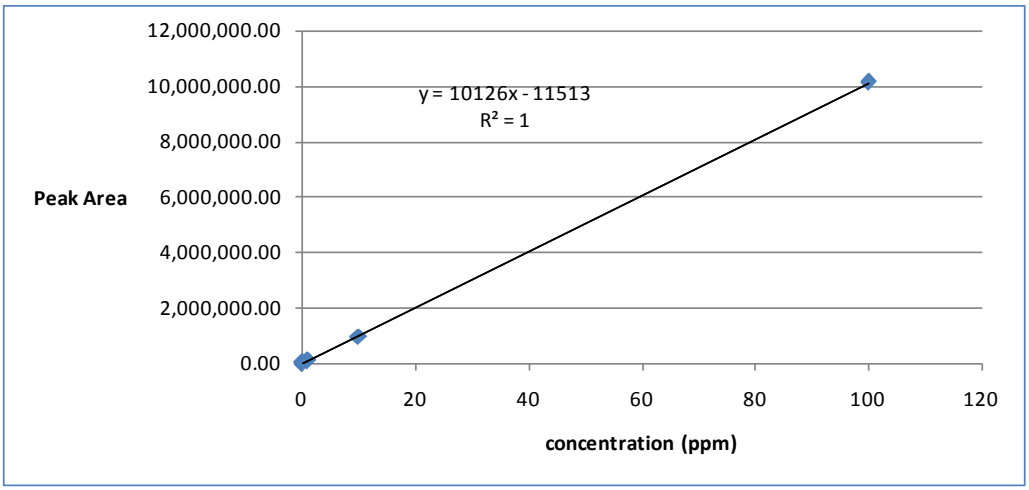

Figure 2. Calibration curve of *trans*-cinnamic acid (CA).

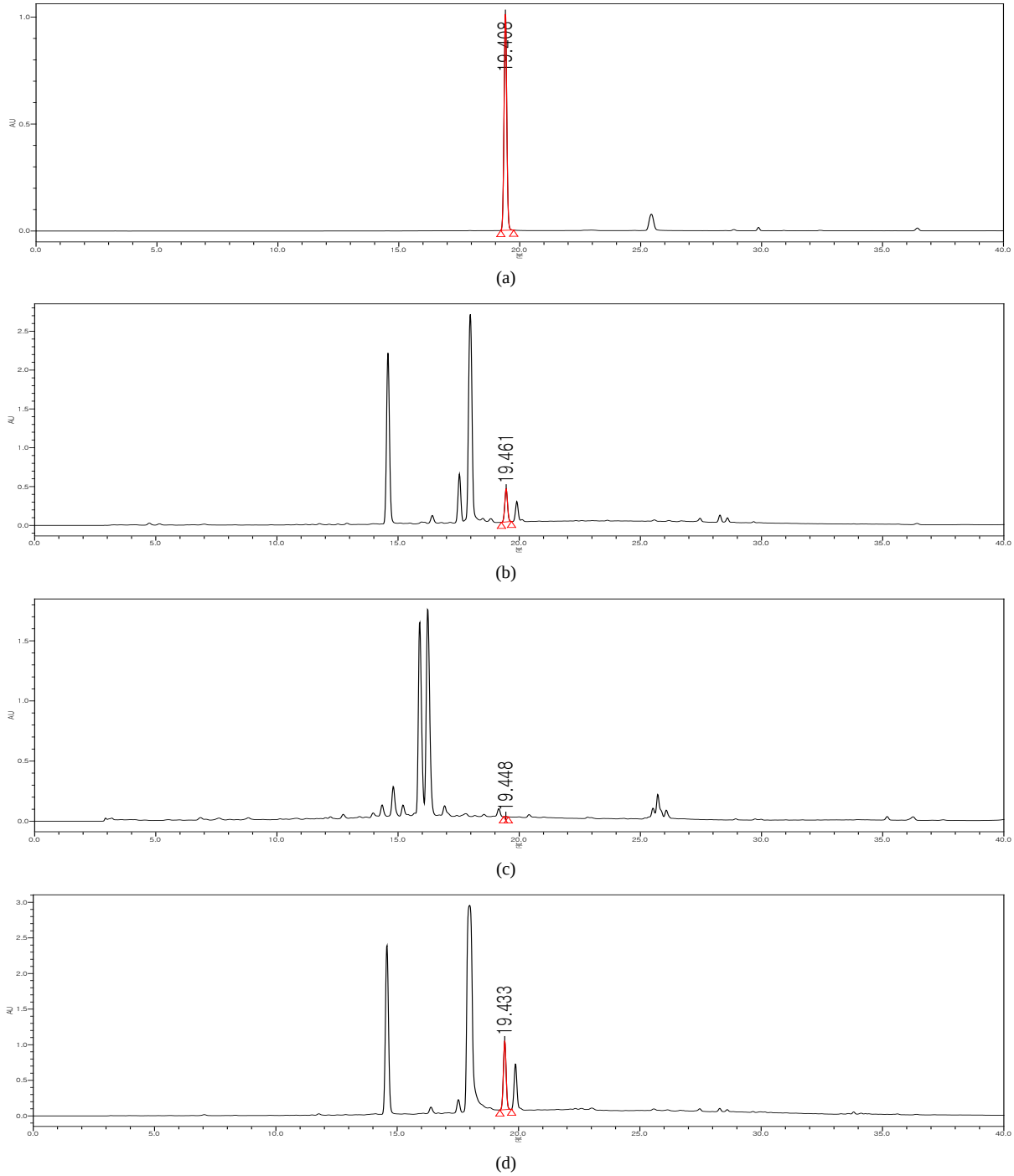

Figure 3. HPLC chromatograms of *trans*-cinnamic acid (CA) (a); The MeOH extract of the bark of *C. loureirii* (b); The leaf of *C. loureirii* (c); and Cinnamon powder A (d).

products such as breakfast cereal, bakery products, desserts, crisp bread, and tea. In addition, gas chromatography-flame ionization detection (GC-FID) was used to quantitatively determine the concentrations of cinnamaldehyde, cinnamylalcohol, and salicylaldehyde in cinnamon contained in biopesticides [25] [26]. Moreover, the therapeutic potential of CA has been reported. For example, CA and its derivatives have been used for α-glucosidase inhibition, and CA showed that anti-auxin effects in plants can be utilized as pesticides [15] [27] [28]. The CA contents in the *Cinnamomum* spp. extracts and in the cinnamon powder were quantified by using a

Table 1. *trans*-Cinnamic acid (CA) contents of the MeOH extracts of *Cinnamomum* spp.

Sample		CA (mg/g extract)
C. loureirii	Bark[1]	16.97 ± 0.11
	Leaf[2]	2.50 ± 0.25
C. camphora	Bark[2]	0.31 ± 0.02
	Leaf[2]	0.26 ± 0.01
	Heartwood[2]	0.69 ± 0.06
C. japonicum	Bark[2]	0.23 ± 0.02
	Leaf[2]	0.10 ± 0.02
	Heartwood[2]	0.10 ± 0.01

Data is represented as the mean ± SD (n = 3) in mg/g of the extract. [1]The bark of *C. loureirii* was purchased from Omniherb. [2]The MeOH extracts were purchased from KRIBB.

Table 2. *trans*-Cinnamic acid (CA) contents of the MeOH extracts in commercially produced cinnamon powder.

Sample	CA (mg/g extract)
Cinnamon powder A	47.60 ± 1.34
Cinnamon powder B	37.76 ± 1.00
Cinnamon powder C	22.87 ± 0.84

Data is represented as the mean ± SD (n = 3) in mg/g of the extract.

calibration curve. Its data for the extracts confirmed a good linear relationship, and the resulting equations were operational in the concentration range. The linear calibration equation is Y = 10,126 X − 11,513, where Y is the peak area and X is the content of CA. The correlation coefficient (r^2) was 1 (**Figure 2**).

The contents of CA in *Cinnamomum* spp. and in commercially available cinnamon powder were determined by using HPLC. Our results demonstrated that this simple method gives rapid and accurate results. And our results can be utilized as a guideline for analysis of CA in *Cinnamomum* spp. and its powders.

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