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Phenolic Constituents in the Fruits of *Cinnamomum zeylanicum* and Their Antioxidant Activity

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Defatted cinnamon fruit powder was successively extracted with benzene ethyl acetate, acetone, MeOH, and water. The concentrated water extract contained the maximum amount of phenolics and showed the highest antioxidant activities. Hence, it was fractionated by Diaion HP-20SS, Diaion HP-20, and Sephadex LH-20 column chromatographies. It gave five purified compounds, the purities of which were analyzed by HPLC. Compounds **1**−**5** were identified as 3,4-dihydroxybenzoic acid (protocatechuic acid), epicatechin-(2β→*O*-7,4β→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-1), 4-[2,3-dihydro-3-(hydroxymethyl)-5-(3-hydroxypropyl)-7-(methoxy)benzofuranyl]-2-methoxyphenyl β-D-glucopyranoside (urolignoside), quercetin-3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (rutin), and quercetin-3-*O*-α-L-rhamnopyranoside by using extensive spectral studies. The antioxidant activities of purified compounds were screened for their antioxidative potential using β-carotene−linoleate and 1,1-diphenyl-2-picrylhydrazyl model systems. All of the compounds showed antioxidant and radical scavenging activities. This is the first report of the isolation and identification of nonvolatile constituents and as well as antioxidant activities from cinnamon fruits.

KEYWORDS: ***Cinnamomum zeylanicum*; fruits; phenolics; protocatechuic acid; cinnamtannin B-1; urolignoside; rutin; quercetin; antioxidant activity**

INTRODUCTION

Antioxidants stabilize polyunsaturated fatty acids in foods by reacting with free radicals, chelating metal ions, and interrupting the propagation phase of lipid oxidation. The most widely used synthetic antioxidants are butylated hydroxyanisole (BHA), propyl gallate, and 2-*tert*-butylhydroquinone (TBHQ) to prevent the oxidation of lipids in foods (*1*). Even though functions of natural and synthetic antioxidants are similar, questions have been raised concerning the safety of some of the commercial antioxidants because model studies indicated mutagenesis and carcinogenesis associated with some synthetic antioxidants (*2*). Therefore, the extraction, characterization, and utilization of natural antioxidants are of considerable interest (*3*, *4*).

Natural antioxidants such as tocopherols, ascorbic acid, and flavonoids have gained the interest of consumers, scientists, and medical and pharmaceutical industries because of their antitumor, antimutagenic, and anticarcinogenic activities. Among them plant phenolics constitute one of the major classes of natural antioxidant, and the antioxidant properties of certain plant phenols have been well established (*4*). These phenolics occur in all parts of the plant including fruits, vegetables, nuts, seeds, leaves, flowers, roots, and barks. Flavonoids are widely distributed and have been reported to act as antioxidants in biological systems (*5*). Many leguminous plants such as soybeans, sesame seeds, and rosemary and spices contain significant levels of flavonoids, the antioxidant activities of which are comparable to those of α-tocopherol, vitamin E, and many synthetic antioxidants. Flavonoids are some of the most common and active naturally occurring antioxidant compound groups used in food, because of their activity in both hydrophilic and lipophilic systems (*6*).

Barks of *Cinnamomum* plants are used as spice and herbal medicine, and *Cinnamomum zeylanicum* bark contains dimeric, trimeric, and higher oligomeric proanthocyandins with doubly linked bis-flavan-3-ol units in the molecule. This class of proanthocyanidins is known to occur widely in common foods (*7*) as well as the singly linked proanthocyanidins. In contrast to barks, cinnamon fruits have not been studied well, and little is known about their nonvolatile components because they are untouched natural resources. A systematic investigation on the chemical composition of the fruits was, therefore, required for utilization of them in the food industry. Earlier the chemical composition of the volatiles from the fruits and fruit stalks and their bioactivities were investigated by this laboratory (*8*−*13*).

In the present paper, the isolation of chemical constituents from the water extract of cinnamon fruits and their identification using spectral data are reported. Furthermore, antioxidant

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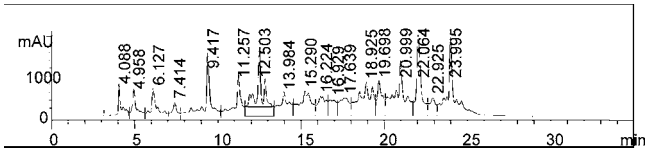


Figure 1. HPLC chromatogram of water extract using a C_{18} column (250 × 4.6 mm i.d., 5 μm particle size), detection at 280 nm, mobile phase of 1% acetic acid in water (A) and MeOH (B), flow rate of 0.7 mL/min, linear gradient from 5 to 50% B in A for 0–25 min and 5% B in A at 25–30 min followed by 5 min of equilibrium with 5% B.

activities of the isolated compounds were studied in different in vitro model systems. This is the first report of the isolation and identification of chemical composition and as well as antioxidant activities from cinnamon fruits.

MATERIALS AND METHODS

Equipment. Mass spectra were recorded on JMS SX-102A and HX-110/110A (JEOL, Tokyo, Japan) for fast atom bombardment (FAB) and liquid secondary ion mass spectrometry (LSIMS), respectively. LCQ Classic (an ion trap mass spectrometer, Thermoelectron, Waltham, MA) and Apex II 70e (a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers (Bruker Daltonics, Billerica, MA) were equipped with syringe pumps for electrospray ionization (ESI) mass spectrometries. IR spectra were recorded on a Bruker-IFS 25 spectrometer using KBr disks. NMR spectra were recorded on Bruker DRX 600 and AVANCE 800 instruments (Karlszruhe, Germany). Tetramethylsilane was used as an internal standard.

Extraction. Cinnamon fruits were dried at 30 °C in the shade for 1 week. The dried fruits were powdered (60−80 mesh) and extracted (510 g) in a Soxhlet extractor with 1500 mL of hexane for 6 h. The extract was filtered and concentrated at 35 °C under vacuum to get 9.60 g of yield. The defatted fruit powder (500 g) was extracted successively in the Soxhlet extractor for 4 h with solvents such as benzene, ethyl acetate, acetone, MeOH, and water (each 1500 mL). The extracts were concentrated under reduced pressure at 35 °C to remove solvents. The yields of benzene, ethyl acetate, acetone, MeOH, and water extracts were 3.90, 9.35, 8.70, 24.95, and 19.35 g, respectively.

Fractionation. Part of the water extract was redissolved in 10 mL of water subjected to HPLC analysis (**Figure 1**) and transferred to a column (60 cm × 30 mm i.d.) filled with Diaion HP-20SS resin (80 g, Mitsubishi Chemical, Tokyo, Japan). Elution was initiated with 100% water and continued by increasing the proportion of MeOH, and final elution was with acetone. A total of 20 fractions were collected (200 mL each) (**Scheme 1**).

HPLC Analysis. Each fraction was analyzed using an HPLC system (HP 1100, Agilent, CA) fitted with an ODS column (Zorbax C_{18}, 250 × 4.6 mm, 5 μm, Agilent). The gradient mobile phase consisted of 1% acetic acid in water (A) and MeOH (B) with the flow rate of 0.7 mL/min. The elution program involved a linear gradient from 5 to 50% B in A for 0−25 min and 5% B in A by 25−30 min followed by 5 min of equilibrium with 5% B. The eluted compounds were detected by their absorbance at 280 nm. Fractions with similar elution profiles were combined and concentrated under reduced pressure before lyophilization.

Isolation of Compounds 1−5. A part of the water-eluted fractions was subjected to the Diaion HP-20 column (80 g, length 60 cm × 30 mm i.d.) to obtain compound **1** (27.9 mg). Fractions eluted by H_2O/MeOH (8:2) (**Scheme 1**) were subjected to repeated column chromatography on the HP-20 (20 g, H_2O and MeOH mixtures) and Sephadex LH-20 columns (10 g, Amersham Biosciences, Piscataway, NJ; H_2O and MeOH mixtures) to get compound **2** (16.33 mg). The water/MeOH (7:3) eluted fraction was subjected to repeated column chromatography on the HP-20 (20 g, H_2O and MeOH mixtures) and Sephadex LH-20 columns (7 g, H_2O and MeOH mixtures) to obtain compounds **3** (26 mg), **4** (9.2 mg), and **5** (19.2 mg). The purity of compounds **1**−**5** was analyzed by HPLC (the same conditions as mentioned above), and they showed single peaks at retention times of 9.81, 13.55, 20.04, 22.91, and 24.73 min, respectively (**Figure 2**).

Identification. Compound **1** was a colorless crystalline powder, mp 200−201 °C. It gave bluish green color with ferric chloride (*10*). ^{1}H and ^{13}C NMR data are presented in **Table 1**. FAB and ESI mass spectra in the negative-ion mode showed a peak at *m*/*z* 153 for $[M - H]^-$. Triethanolamine was used as the matrix for FAB mass spectrometry. The DMSO solution of compound **1** was diluted by 50% aqueous MeOH and introduced into the ion-trap mass spectrometer.

Compound **2** was a brown amorphous powder. It showed dark green color with ferric chloride and pink color on heating with 2 N HCl, $[\alpha]^{25}_D$ 65.1° (*c* 0.5 in acetone). ^{1}H and ^{13}C NMR spectral data are presented in **Table 2**. LSIMS spectra showed $[M + H]^+$ and $[M - H]^-$ at *m*/*z* 865.2 and 863.1, respectively. Magic bullet, a mixture of dithiothreitol and dithioerythritol (3:1), was used as the matrix for LSIMS.

Compound **3** was an amorphous powder; it showed bluish green color with ferric chloride reaction. It was positive for the Molish test (*10*), $[\alpha]^{25}_D$ −30.1° (*c* 0.5 in MeOH). ^{1}H and ^{13}C NMR spectral data are presented in **Table 3**. LSIMS gave deprotonated ion at *m*/*z* 521 in the negative-ion mode. Triethanolamine was used as the matrix. The pyridine solution of compound **3** was diluted by MeOH and introduced into the FT-ICR mass spectrometer.

Compound **4** was obtained as pale yellow crystals, mp 214−215 °C. It gave a dark green color with ferric chloride reaction. It showed positive for the Shinoda test (*10*). UV (MeOH) λ_{max} nm 260, 268 (sh), 298 (sh), 360; $AlC1_3$ 275, 303 (sh), 433; $AlCl_3$ + HCl 271, 300, 364 (sh), 402; NaOMe 272, 327, 410; NaOAc 272, 325, 393; NaOAc + H_3BO_3 262, 298, 388. ^{1}H and ^{13}C NMR data are presented in **Table 4**. LSIMS mass spectrum showed protonated molecule peak at *m*/*z* 611.2, and the product ions from $[M + H]^+$ ion were observed at *m*/*z* 465 and 303 by linked scan at constant *B*/*E*. Glycerol was used for the matrix.

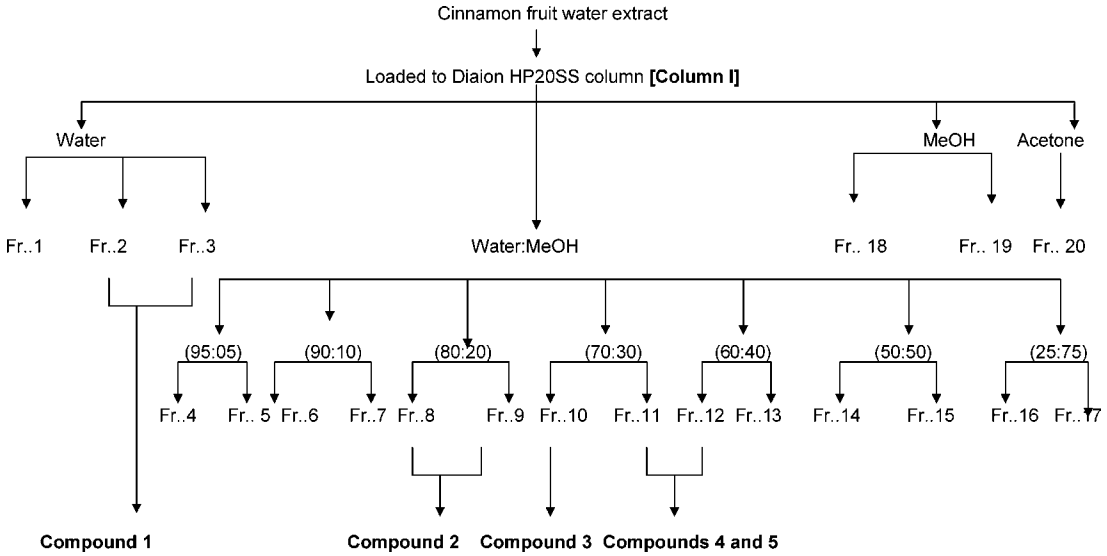
Compound **5**, pale yellow crystals, mp 250−252 °C, showed a dark green color with ferric chloride reaction and pink color with the Shinoda test. UV (MeOH) λ_{max} nm 257, 266 (sh), 301 (sh), 350; $AlC1_3$ 277, 303 (sh), 333, 430; $AlCl_3$ + HCl 272, 303 (sh), 354, 402; NaOMe 272, 329, 394; NaOAc 273, 323 (sh), 372; NaOAc + H_3BO_3 262, 298 (sh), 368. ^{1}H and ^{13}C NMR data are presented in **Table 5**. LSIMS mass spectra showed $[M + H]^+$ at *m*/*z* 449.1, and the product ion was observed at *m*/*z* 303.1 in the linked scan measurement. Magic bullet was used as the matrix.

Sample Preparation for Antioxidant Assay. Five milligrams of each compound, namely, protocatechuic acid, cinnamtannin B-1, urolignoside, rutin, and quercetin-3-*O*-α-L-rhamnopyranoside, was dissolved in MeOH and made up to 5 mL with MeOH.

Antioxidant Assay using b-Carotene−Linoleate Model System. The antioxidant activities of pure compounds **1**−**5** and BHA were evaluated according to the method of Jayaprakasha and Jaganmohan Rao (*14*). Amounts of 0.2 mg of β-carotene, 20 mg of linoleic acid, and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed in 0.5 mL of chloroform. Chloroform was removed at 40 °C under vacuum using rotary evaporator. The resulting mixture was immediately diluted with 10 mL of triple-distilled water and was mixed well for a 1−2 min. The emulsion was further made up to 50 mL with oxygenated water. Four milliliter aliquots of the β-carotene emulsion were pipetted into different test tubes containing pure compounds **1**−**5** and BHA (equivalent to 50 and 100 ppm) in MeOH. BHA was used for comparison purposes. A control sample containing 0.2 mL of MeOH and 4 mL of the above emulsion was prepared. The tubes were placed at 50 °C in a water bath, and the optical density at 470 nm was measured at zero time ($t = 0$). The measurement of optical density was recorded at intervals of 30 min and until the color of β-carotene disappeared in the control tubes ($t = 180$ min). A mixture prepared as above without β-carotene served as the blank. All determinations were carried out in triplicate and averaged.

The antioxidant activity (AA) of the compounds **1**−**5** and BHA was evaluated in terms of bleaching of the β-carotene using the following formula $AA = 100[1 - (A_0 - A_t)/(A^\circ_0 - A^\circ_t)]$, where A_0 and A°_0 are the

Scheme 1. Fractionation of Water Extract of Cinnamon Fruits Using the HP-20 Column



absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A_t° are the absorbance values measured in the test sample and control, respectively, after incubation for 180 min.

Radical Scavenging Activity Using the DPPH Method. Different concentrations of pure compounds **1**−**5** (12.5 and 25 ppm) and BHA (12.5 and 25 ppm) were taken in different test tubes. The sample volume was adjusted to 0.1 mL by adding MeOH. Five milliliter aliquots of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) were added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min (*15*). The control was prepared as above without any sample or BHA. MeOH was used for the baseline correction. The changes in the optical density (OD) of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and calculated using the following formula: % radical scavenging activity = (control OD − sample OD/control OD) × 100.

RESULTS AND DISCUSSION

C. zeylanicum fruits were powdered and successively extracted with hexane, benzene, ethyl acetate, acetone, methanol, and water. The maximum and minimum yields were obtained with methanol and benzene extracts, respectively, with respect to dried fruits (w/w). The water extract showed the maximum phenolic content and the highest antioxidant activities as compared to the other extracts. The HPLC chromatogram of water extract showed several peaks (**Figure 1**) at 280 nm indicating the presence of several phenolic compounds. The water extract was fractionated on Diaion HP-20SS (**Scheme 1**), and these fractions were further chromatographed and purified on Diaion HP-20SS, Diaion HP-20, and Sephadex LH-20 columns, which afforded five major components, compounds **1**−**5**. The purity of each compound was analyzed by HPLC, and the chromatograms are presented in **Figure 2**.

Compound **1** gave positive reaction for ferric chloride, indicating the presence of phenolic hydroxyl groups. It also gave effervescence with dilute sodium bicarbonate, indicating the presence of a carboxyl group. The ^{1}H NMR spectrum of this compound (**Table 1**) showed signals at δ 7.27 (dd; 1.8, 8.2 Hz), 6.74 (d, 8.2 Hz), and 7.33 (d, 1.8 Hz), which indicated the presence of a 1,3,4-trisubstituted benzene ring. The ^{13}C NMR spectrum showed the presence of seven carbons. The signal at δ 167.6 is assigned to carboxyl carbon (COOH). The four signals at δ 121.8, 116.6, 115.1, and 122.0 are assigned to C-1 (linked to carboxyl group), C-2, C-5, and C-6, respectively, by HSQC and HMBC. The signals at δ 144.8 and 149.9 are assigned to the C-3 and C-4 carbons linked to hydroxyl groups. Thus, compound **1** was identified as 3,4-dihydroxybenzoic acid (protocatechuic acid) (**Figure 3**), and ^{13}C NMR chemical shifts of compound **1** matched with the values reported (*16*).

Compound **2** was isolated as a brown amorphous powder and developed a dark green color with ferric chloride, indicating it to be a phenolic compound. It gave pink color on heating with 2 N methanolic hydrochloric acid and UV absorption maxima (λ_{max}) at 223 and 280 nm, indicating it to be a proanthocyanidin. The LSIMS spectrum of compound **2** showed the $[M + H]^+$ ion at *m*/*z* 865.2, and the product ions were observed at *m*/*z* 713 ($[M + H - 152]^+$), 575 ($[M + H - 290]^+$), and 287. In the negative-ion mode, the $[M - H]^-$ ion was observed at *m*/*z* 863.1, and the product ions were observed at *m*/*z* 711, 573, 579, 531, 451, and 411. The fragmentation patterns indicated that this compound was a triflavanoid with an A-type linkage between the top and middle units (*17*). The NMR chemical shifts and their assignments of compound **2** have been presented in **Table 2**. The ^{1}H NMR spectrum confirmed the presence of an A-type doubly linked proanthocyanidin unit by the pair of doublets at δ 3.27 and 4.13 with J = 3.4 Hz, which were assigned to H-3 and H-4 of the C ring. The A-type linkage was also supported by the presence of a ketal carbon signal at δ 99.9 for the C-2 position of the C ring (*18*). Three carbon signals at δ 67.2, 72.6, and 67.5 correspond to sp^3 carbons linked to hydroxyl groups, that is, C-3 carbons of rings C, F, and I, respectively, present in three proanthocyanidin units (*19*). By a combination of DQF-COSY, HSQC, and HMBC, a planar structure was established. The linkages between the three proanthocyanidin units were confirmed by HMBC from H-4 of ring C to C-7, -8, and -9 of ring D, from H-3 of ring F to C-8 of ring G, and from H-4 of ring F to C-7, and -8 of ring G. The presence of *ent*-epicatechin or epicatechin units was suggested by signals at δ 78.9 and 80.3 of the flavan C-2 on rings F and I. On the basis of the above spectral data compound **2** was characterized as (*ent*-)epicatechin-(2→O-7,-4→8)-(*ent*-)epicatechin-(4→8)-(*ent*-)epicatechin. The ^{1}H and ^{13}C NMR spectra of compound **2** agreed with those of epicatechin-(2β→O-7,4β→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-1) (*18*), and HPLC analysis of compound **2** and authentic

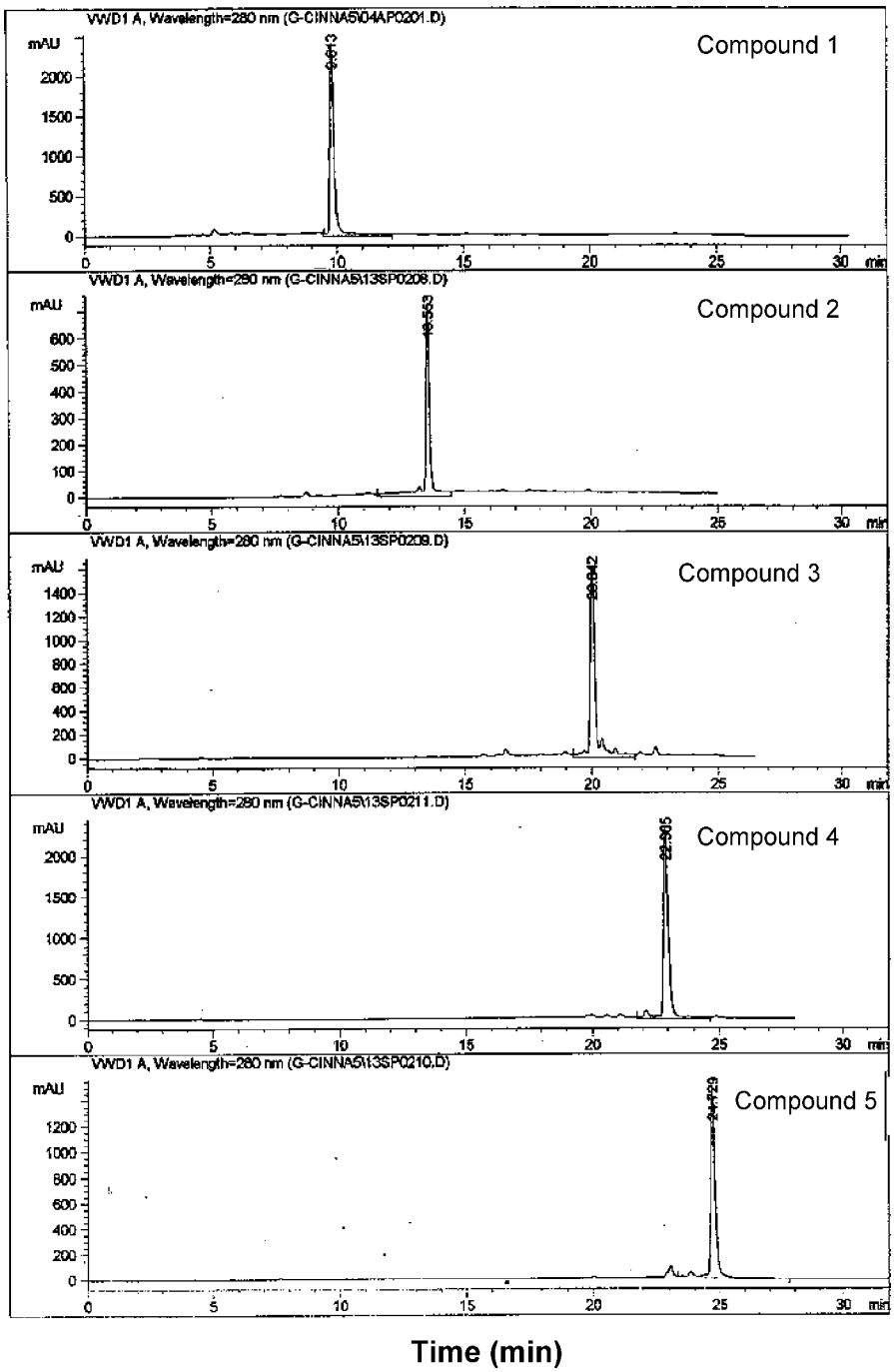


Figure 2. HPLC chromatograms of compounds **1**–**5**.

Table 1. ^{1}H and ^{13}C NMR (DMSO-d_6) Chemical Shifts of **1**

H/C	^{1}H δ^{a}	multiplicity	coupling constants (*J*, Hz)	^{13}C δ^{b}
1				121.8
2	7.33	d	1.8	116.6
3				144.8
4				149.9
5	6.74	d	8.2	115.1
6	7.27	dd	1.8, 8.2	122.0
COOH				167.6

[a] At 600 MHz. [b] At 150.9 MHz.

cinnamtannin B-1 confirmed their identity. Hence, compound **2** is identified as cinnamtannin B-1 (**Figure 3**).

Compound **3** gave positive results for ferric chloride and Molish test, indicating the presence of phenolic groups and a sugar moiety. The UV spectrum in MeOH showed the λ_{max} at 227 and 278 nm, which indicated the presence of an aromatic system. The IR spectrum of compound **3** showed peaks at 3345, 1604, and 1517 cm^{-1}, which indicated the presence of hydroxyl and aromatic rings. LSIMS spectra of compound **3** showed the deprotonated ion peak at *m*/*z* 521.1. The product ions were observed at *m*/*z* 359 ([M − H − 162]$^{-}$) and 341 ([M − H − 180]$^{-}$), indicating the presence of hexose in the molecule. In the positive-ion mode, the molecular ion peak was observed at *m*/*z* 522.3 instead of [M + H]$^{+}$ and the product ions were observed at *m*/*z* 360, 342, and 331. The ESI FT-ICR mass spectrum showed [M + Na]$^{+}$ at *m*/*z* 545.1996, which was in good agreement with the calculated value of 545.1993 for $C_{26}H_{34}O_{11}Na$ (0.5 ppm error).

The ^{1}H NMR signals at δ 1.81 (2H, m), 2.61 (2H, t, 7.5 Hz), and 3.56 (2H, t, 6.5 Hz) of compound **3** indicated the structure

Table 2. ^{1}H and ^{13}C NMR Data of **2** in CD_3OD

H/C	^{1}H chemical shift	multiplicity	J_{H-H} (Hz)	^{13}C chemical shift	HMBC
2				99.9	
3	3.27	1H, d	3.4	67.2	first C-10
4	4.13	1H, d	3.4	28.9	first C-2, 3, 5, 9, 10 second C-7, 8, 9
5				154.2	
6	6.00	1H, d	2.3	96.6	first C-5, 7, 8, 10
7				157.9	
8	5.95	1H, d	2.3	98.3	first C-6, 7, 9, 10
9				156.8	
10				104.9	
1′				132.5	
2′	7.02	1H, d	2.0	115.8	first C-2, 1′, 3′, 4′, 6′
3′				145.5	
4′				146.6	
5′	6.81	1H, d	8.2	115.7	first C-1′, 3′, 4′
6′	6.84	1H, dd	2.1, 8.2	119.9	first C-2, 2′, 4′
2	5.69	1H, s		78.9	second C-1′, 2′, 6′
3	4.11	1H, br s		72.6	second C-2, 10 third C-8
4	4.55	1H, br s		38.3	second C-2, 3, 5, 9, 10 third C-7, 8
5				155.8	
6	5.79	1H, s		96.1	second C-5, 7, 8, 10
7				151.1	
8				106.4	
9				151.8	
10				106.7	
1′				131.8	
2′	7.30	1H, d	1.9	116.7	second C-2, 3′, 4′, 6′
3′				145.9	
4′				146.3	
5′	6.83	1H, d	8.2	116.1	second C-1′, 3′, 4′
6′	7.18	1H, dd	1.9, 8.2	121.4	second C-2, 2′, 4′
2	4.37	1H, s		80.3	third C-3, 1′, 2′, 6′
3	3.85	1H, br s		67.5	
4	2.82	2H, m		29.9	third C-2, 3, 9, 10
5				156.1	
6	6.09	1H, s		96.4	third C-5, 7, 8, 10
7				155.6	
8				108.9	
9				155.8	
10				100.0	
1′				133.2	
2′	6.81	1H, d	1.8	115.5	third C-2, 1′, 3′, 4′, 6′
3′				145.8	
4′				145.3	
5′	6.74	1H, d	8.2	116.0	third C-1′, 3′
6′	6.71	1H, dd	1.8, 8.2	119.4	third C-2, 2′, 4′

of $CH_2CH_2CH_2O$, and signals at 3.82 (3H, s) and 3.85 (3H, s) indicated the presence of two aromatic methoxyl groups the ^{13}C NMR signals of which are assigned to those at δ 56.7 and 56.8, respectively, by HSQC (**Table 3**). The ^{1}H NMR also showed the presence of three aromatic proton signals at δ 7.12 ($J = 8.4$ Hz), 6.91 (dd, $J = 1.9$, 8.4 Hz), and 7.02 (d, $J = 1.9$ Hz) characteristic of a 1,3,4-trisubstituted benzene ring. In addition, two more aromatic signals at δ 6.71 (1H, s) and 6.72 (1H, s), indicating the presence of another aromatic system with four-carbon substitution, was detected. HMBC from the methoxy protons was used for assignment of the carbons to which the methoxy groups attached, and it was revealed that the two methoxy groups were on different aromatic rings. The HMBC experiment also showed attachment of the $CH_2CH_2CH_2O$ structure on the latter aromatic system and connection between the two aromatic systems via a fused heterocyclic ring system. The presence of a hexose assumed from the MS analysis was verified by an anomeric signal at δ 4.88 on the ^{1}H NMR spectrum and at δ 102.9 on the ^{13}C NMR spectrum. From

Table 3. ^{1}H and ^{13}C NMR Data of **3** in CD_3OD

H/C	^{1}H chemical shift	multiplicity	J_{H-H} (Hz)	^{13}C chemical shift	HMBC
1				137.1	
2	6.72	1H, br s		114.2	C-1, 3, 4, 6, 7
3				145.3	
4				147.5 or 147.6	
5				129.6	
6	6.71	1H, br s		117.9	C-2, 3, 4, 7, 8′
7	2.61	2H, t	7.7	32.9	C-1, 2, 6, 8, 9
8	1.80	2H, m		35.8	C-1, 7, 9
9	3.56	2H, t	6.5	62.2	C-7, 8
3-*O*-Me	3.85	3H, s		56.8	C-3
1′				138.4	
2′	7.02	1H, d	2.0	111.2	C-1′, 3′, 4′, 6′, 7′
3′				150.9	
4′				147.5 or 147.6	
5′	7.13	1H, d	8.4	118.0	C-1′, 3′, 4′
6′	6.92	1H, dd	2.0 8.4	119.4	C-2′, 4′, 7′
7′	5.54	1H, d	5.9	88.5	C-4, 5, 1′, 2′, 6′, 8′, 9′
8′	3.44	1H, m		55.7	C-4, 5, 6, 1′, 7′, 9′
9′	3.74	1H, dd	7.6, 11.1	65.1	C-5, 7′, 8′
	3.83	1H, dd	4.1, 11.1		C-5, 7′, 8′
3′-*O*-Me	3.82	3H, s		56.7	C-3′
1″	4.88	1H, d	7.6	102.8	C-4′
2″	3.48	1H, dd	7.6, 9.2	74.9	C-1″, 3″
3″	3.45	1H		77.8	C-2″, 4″
4″	3.38	1H		71.3	C-3″, 5″
5″	3.38	1H		78.2	C-6″
6″	3.67	1H, br d	12.6	62.5	C-5″
	3.85	1H, br d	12.6		C-4″

Table 4. ^{1}H and ^{13}C NMR Data of **4** in CD_3OD

H/C	^{1}H chemical shift	multiplicity	J_{H-H} (Hz)	^{13}C chemical shift	HMBC
2				159.3	
3				135.6	
4				179.4	
5				163.0	
6	6.20	1H, d	2.1	100.0	C-5, 7, 8, 10
7				166.3	
8	6.39	1H, d	2.1	94.9	C-6, 7, 9, 10
9				158.6	
10				105.6	
1′				123.1	
2′	7.66	1H, d	2.2	117.7	C-2, 1′, 3′, 4′, 6′
3′				145.9	
4′				149.8	
5′	6.87	1H, d	8.4	116.1	C-1′, 3′, 4′
6′	7.62	1H, dd	2.2, 8.4	123.5	C-2, 2′, 4′
1″	5.10	1H, d	7.7	104.7	C-3, 5″
2″	3.46	1H, dd	7.7, 9.2	75.7	C-1″, 3″
3″	3.40	1H, m		78.2	C-2″, 4″
4″	3.25	1H, m		77.2	C-3″, 5″, 6″
5″	3.31	1H, m		71.4	C-1″, 3″, 4″, 6″
6″	3.38	1H, m		68.6	C-4″, 5″, 1‴
	3.79	1H, dd	1.5, 11.1		C-4″, 1‴
1‴	4.51	1H, d	1.5	102.4	C-6″, 2‴ and/or 3‴, 5‴
2‴	3.62	1H, dd	1.5, 3.5	72.1	C-3‴, 4‴
3‴	3.53	1H, dd	3.5, 9.5	72.2	C-2‴, 4‴
4‴	3.27	1H, m		73.9	C-2‴ and/or 3‴, 5‴
5‴	3.43	1H, m		69.7	C-3‴, 4‴
6‴	1.11	3H, d	6.2	17.9	C-4‴, 5‴

chemical shifts of ^{1}H and ^{3}C NMR and coupling constants of the ^{1}H signals the hexose was determined as β-glucopyranose. The NMR spectral data of compound **3** shown in **Table 3** well matched those of urolignoside (*20*) with some minor difference in assignment. A coupling constant between H-7′ and H-8′ of 5.9 Hz indicated the trans configuration, and the absolute configuration of C-7′ and C8′ was determined to be 7′*S*,8′*R*

Table 5. ^{1}H and ^{13}C NMR Data of **5** in CD_3OD

H/C	^{1}H chemical shift	multiplicity	J_{H-H} (Hz)	^{13}C chemical shift	HMBC
2				159.3	
3				136.2	
4				179.7	
5				163.2	
6	6.20	1H, d	2.1	99.8	C-5, 7, 8, 10
7				165.9	
8	6.37	1H, d	2.1	94.7	C-4, 6, 7, 9, 10
9				158.5	
10				105.9	
1′				123.0	
2′	7.33	1H, d	2.1	116.9	C-2, 3′, 4′, 1′ and/or 6′
3′				146.4	
4′				149.8	
5′	6.90	1H, d	8.3	116.4	C-2, 2′, 3′, 4′, 1′ and/or 6′
6′	7.30	1H, dd	2.1, 8.3	122.9	C-2, 2′, 3′, 4′
1″	5.34	1H, d	1.6	103.6	C-3, 2″, 3″
2″	4.21	1H, dd	1.6, 3.3	71.9	C-3″, 4″
3″	3.74	1H, dd	3.3, 9.6	72.1	C-2″, 4″
4″	3.33	1H, dd	9.6, 9.6	73.3	C-3″, 5″
5″	3.41	1H, dq	6.2, 9.6	72.0	C-3″, 4″
6″	0.93	3H, d	6.2	17.7	C-4″, 5″

(**Figure 3**), by comparison with the ^{13}C NMR chemical shifts with those of the two diastereomers (*21*).

Compounds **4** and **5** gave positive ferric chloride reaction and pink color in the Shinoda test, which indicated the presence of a flavonoid residue with hydroxyl groups. Both compounds were positive in the Molish test, which suggested the presence of a sugar moiety. The UV spectra of compounds **4** and **5** in MeOH showed λ_{max} peaks at 259, 266 (sh), 299 (sh), and 359 nm. The bathochromic shift of about 51 and 49 nm without decreasing the intensity of band I by the addition of sodium methoxide indicates the presence of a free hydroxyl group at position C-4′ in compounds **4** and **5**, respectively (*22*). The bathochromic shift of band II (12 and 16 nm) with sodium acetate indicates the presence of a free 7-hydroxyl group. Furthermore, the presence of the B ring ortho dihydroxyl group was confirmed by bathochromic shift of band I (28 nm) in the presence of sodium acetate and boric acid. The presence of the 5-hydroxyl group was confirmed by aluminum chloride and hydrochloric acid addition. A stable bathochromic shift of bands I and II with aluminum chloride and aluminum chloride and hydrochloric acid shows the presence of a hydroxyl group at C-3′ or C-5′. The mass spectra of compound **4** showed a protonated molecule at *m*/*z* 611.2 and the product ions at 465 ($[M + H - 146]^+$) and 303 ($[M + H - 146 - 162]^+$). Those of compound **5** showed $[M + H]^+$ at *m*/*z* 449 and the product ion at *m*/*z* 303 ($[M + H - 146]^+$). These results suggested that both compounds included a rhamnosyl residue. Compound **4** had another sugar, hexose, unit connected to rhamnose.

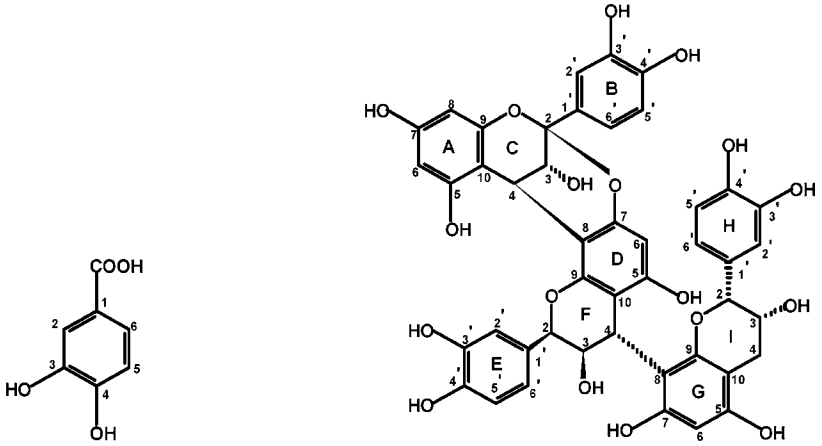
The ^{1}H NMR spectrum of compounds **4** and **5** showed the presence of three aromatic proton signals, which were characteristic of H-5′, H-6′, and H-2′ of the flavonoid B ring with 3′,4′-substitution (**Tables 4** and **5**). Two weakly coupled doublet signals were assigned to two protons on C-6 and C-8 in the A ring. Considering the ^{13}C NMR spectra with carbonyl signals at δ 179.4 and 179.7 in compounds **4** and **5**, respectively, they are assumed to contain a flavonol skelton. As for compound **4**, two anomeric proton signals at δ 4.51 and 5.10 attached to carbons at δ 102.4 and 104.7, respectively, confirmed the presence of disaccharide on the flavonol nucleus. Furthermore, HMBC from the ^{1}H signal at δ 5.10 to carbon at δ 136.6 suggested that the C-3 position of flavonol was glycosylated in compound **4**. A proton signal at δ 1.11 (d, 3H, 6.2 Hz) on the carbon at δ 17.9 indicated one of the sugars had a terminal methyl group. The ^{1}H, ^{13}C, and 2D NMR spectra indicated that the disaccharide is rhamnopyranosyl-(α 1→6)-glucopyranose (rutinose), of which linkage was verified by HMBC from H-6″ to C-1‴ and from H-1‴ to C-6″. Thus, compound **4** was identified as quercetin-3-*O*-rutinoside (rutin, **Figure 3**). The chemical shifts of this compound were matched to reported values (*23*).

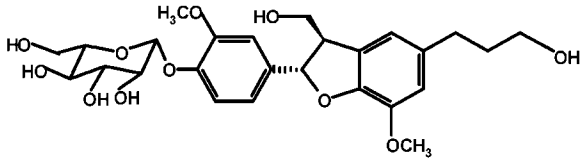
Bathochromic shifts and MS data suggested that compound **5** contained a quercetin skeleton as well as compound **4**, but it has only one anomeric proton at δ 5.34 (d, 1.5 Hz) indicative of the presence of a monosaccharide moiety (**Table 5**). The ^{1}H and ^{13}C NMR spectra confirmed this structure. The presence of a methyl signal at δ 0.93 and coupling constants of ^{1}H NMR, together with ^{13}C NMR chemical shifts, showed that the attached sugar was α-rhamnopyranose. The linkage of the sugar moiety at C-3 of the flavonol skeleton was confirmed from HMBC from the anomeric proton to C-3 and the upfield shift of C-3 signal of quercetin. Thus, compound **5** was identified as quercetin-3-*O*-α-rhamnopyranoside (**Figure 3**), of which chemical shifts were matched to reported values (*24*).

The purified compounds and BHA were dissolved in MeOH and used for antioxidative activity assay using the β-carotene−linolate system. In this system, the inhibition of β-carotene degradation by peroxides arising from oxidation of linoleic acid was measured. Figure 4 shows the antioxidative activity of protocatechuic acid, cinnamtannin B-1, urolignoside, rutin, quercetin-3-*O*-α-L-rhamnopyranoside, and BHA at 50 and 100 ppm concentrations. Protocatechuic acid showed the highest antioxidant activity and urolignoside the lowest by parts per million base comparison. When the activities were compared on a molar basis, cinnamtannin B-1 showed the highest activity followed by rutin and quercetin-3-*O*-α-L-rhamnopyranoside.

Free radical scavenging potentials of the compounds purified from cinnamon fruit extracts and BHA were tested by DPPH assay, and the results are depicted in **Figure 5**. Antioxidants react with DPPH, a stable free radical, and convert it to 1,1-diphenyl-2-picryl hydrazine. The degree of discoloration of the solution by the conversion of DPPH indicates the radical scavenging potential of the sample, which has been mixed with DPPH. Protocatechuic acid, cinnamtannin B-1, urolignoside, rutin, quercetin-3-*O*-α-L-rhamnopyranoside, and BHA showed 77.3, 39.3, 30.4, 44.7, 60.3, and 85% free radical scavenging activities, respectively, at 12.5 ppm concentration. When this activity was compared on a molar basis, cinnamtannin B-1 showed the highest activity followed by rutin and quercetin-3-*O*-α-L-rhamnopyranoside, as in the case of β-carotene antioxidant activity assay.

The radical scavenging activity of phenolic compounds is thought to be attributed to the hydrogen-donating ability of the compounds (*25*). It is well-known that free radicals cause autoxidation of unsaturated lipids in food (*26*). The antioxidants intercept the free radical chain oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming stable end product, which does not initiate or propagate further oxidation of lipid (*27*). In phenolic acids and their esters, radical scavenging activity generally depends on the number of phenolic hydroxyl groups (*28*). In this study cinnamtannin B-1, which has 11 phenolic hydroxyl groups, showed the highest activity on a molar basis. Rutin and quercetin-3-*O*-α-L-rhamnopyranoside, with four phenolic hydroxyl groups, showed similar activity following cinnamtannin B-1. The degree of antioxidant activity of the compounds measured with the β-carotene assay showed





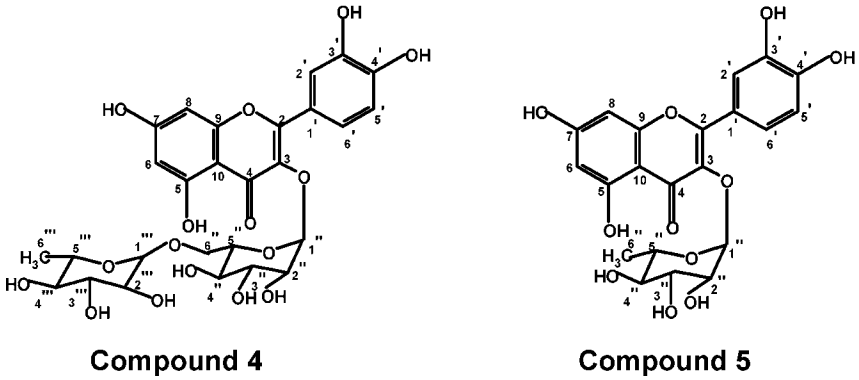


Figure 3. Structures of active compounds **1**–**5** isolated from cinnamon fruits.

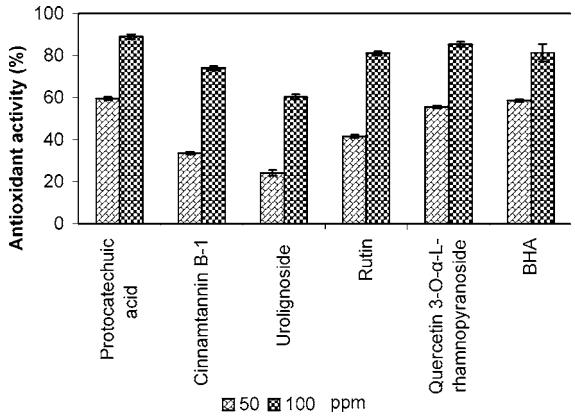


Figure 4. Antioxidant activity of purified compounds from cinnamon fruits using β-carotene–lineoleate model system.

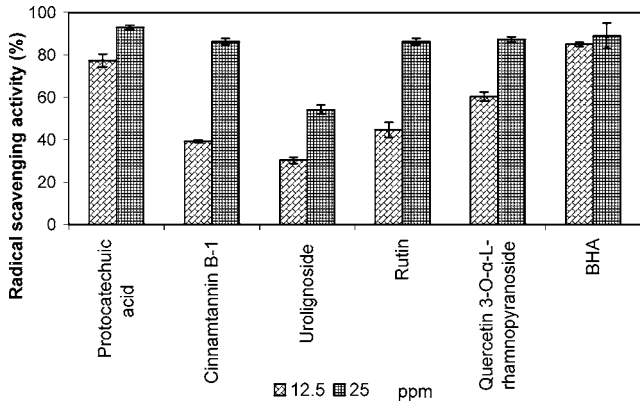


Figure 5. Radical scavenging activity of purified compounds from cinnamon fruits using DPPH method at different concentrations.

a similar tendency, suggesting that the activity is derived from the radical scavenging. The fact that the water extract, which contains the largest amount of phenolics, showed the highest antioxidant and free radical scavenging activities among the cinnamon fruits extracts was, therefore, expected.

In conclusion, cinnamon fruits were extracted with different solvents with increasing polarity. The water extract, which contained the highest amount of phenolics and had the highest antioxidative activity, was subjected to repeated column chromatographies to get five compounds. Compounds **1**−**5** were identified as protocatechuic acid, cinnamtannin B-1, urolignoside, rutin, and quercetin-3-*O*-α-L-rhamnopyranoside, respectively, using extensive NMR and MS analyses. All of the isolated compounds showed potential as antioxidant in β-caro-

tene−linoleate and DPPH model systems. The result of this study revealed the value of the unconventional parts of cinnamon, which have no commercial application at present. This is the first report on nonvolatile constituents from cinnamon fruits. It is expected that further studies can be extended to exploit them for their possible application to the preservation of food products as well as their use as health supplements and neutraceuticals.

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