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Antioxidant Constituents in the Fruits of *Luffa cylindrica* (L.) Roem

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Hydrophilic antioxidant constituents in the fruits of the vegetable *Luffa cylindrica* (L.) Roem (sponge gourds) were separated by an antioxidant-guided assay to yield eight compounds: *p*-coumaric acid (**1**), 1-*O*-feruloyl-*β*-D-glucose (**2**), 1-*O*-*p*-coumaroyl-*β*-D-glucose (**3**), 1-*O*-caffeoyl-*β*-D-glucose (**4**), 1-*O*-(4-hydroxybenzoyl)glucose (**5**), diosmetin-7-*O*-*β*-D-glucuronide methyl ester (**6**), apigenin-7-*O*-*β*-D-glucuronide methyl ester (**7**), and luteolin-7-*O*-*β*-D-glucuronide methyl ester (**8**). The eight compounds were isolated by high-speed countercurrent chromatography and identified by electrospray ionization−mass spectrometry and NMR analysis, and the antioxidant activity was evaluated by the radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical. High-performance liquid chromatography analysis showed that a total amount of the eight compounds in the dried gourds without skin was about 1%. The results demonstrate that the consumption of sponge gourds can supply some antioxidant constituents to human body.

KEYWORDS: ***Luffa cylindrica* (L.) Roem; sponge gourd; antioxidant constituents; separation; identification**

INTRODUCTION

Antioxidants in food have received a great amount of attention as being primary preventive ingredients against various diseases (*1*). It has been suggested that ingesting elevated levels of naturally occurring antioxidants, including polyphenolics, may be beneficial to human health (*2*). The fresh fruits of *Luffa cylindrica* (L.) Roem, which is known as sponge gourd, are a daily vegetable, which is cultivated in the tropical and subtropical Asian regions. Usually, the skin of the gourd is peeled off when it is used as a vegetable. The seeds and sponge of the old fruits are also used in traditional Chinese medicine as an anthelmintic, stomachic, and antipyretic phytomedicinal drug. A very recent report shows that the water extracts from fresh sponge gourds exhibited over 80% inhibition on NO generation stimulated by lipopolysaccharide, an over 40% inhibitory effect on DNA damage induced by SNP in RAW 264.7 macrophage, and a 17.9% scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical, as BHA gave a 94.8% scavenging effect (*3*). In addition, the fresh fruits of *Luffa acutangula*, another species of *Luffa*, showed a certain antioxidant activity (*4*). Previous studies of chemical constituents of this plant mainly involved triterpenoid saponins (*5*−*7*), although apigenin, a flavone glycoside, was isolated from the leaves (*5*). The present study focused on the screening of water soluble antioxidant compounds based on isolation procedures using high-speed countercurrent chromatography (HSCCC) (*8*, *9*), guided by DPPH radical scavenging activity assay. The results yielded eight antioxidant phenolic compounds, which have not yet been reported in the fruit of *L. cylindrica*.

MATERIALS AND METHODS

Materials. One kilogram of freeze-dried sample was prepared from 30 kg of fresh sponge gourds that were purchased in a local store (Hangzhou, China). The fresh sponge gourds were skin-peeled first. Then, the gourds without skin were sliced and freeze-dried to yield 1.03 kg of freeze-dried sample for the following extraction. All solvents for extraction and separation were of analytical grade (Hangzhou Chemicals Inc., China); ascorbic acid and DPPH were purchased from Sigma (Shanghai Division).

Extraction and Preseparation. One kilogram of the freeze-dried sample was extracted two times with 5 L of 90% ethanol for 2 h at 50 °C. The extracts were combined and evaporated to syrup. Defatting with ether and then lyophilizing yielded 141 g of crude extract. A 100 g amount of crude extract was dissolved in 400 mL of water and was subjected to chromatography on a 6 cm i.d. × 1.0 m column filled with 1 kg of AB-8 macroporous gel resin (Chemical Factory of Nankai University, Tianjin, China). Elution was done with increasing solvent strength by using 10, 30, 50, 70, and 90% ethanolic solutions to yield five main fractions: F10 (22 g), F30 (11 g), F50 (17 g), F70 (15 g), and F90 (21 g). Fractions F30 and F50 showed a positive antioxidant activity in the radical scavenging DPPH assay (**Table 1**). F30 and F50 were further fractionated by HSCCC for screening of antioxidant components.

HSCCC Separation. The high-speed countercurrent chromatograph used in the present study was constructed at the Institute of Food and

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Table 1. DPPH Radical Scavenging Capacity of Fractions Recovered from the Ethanol Extract of the Fruits of *L. cylindrica* by Organic Gel Resin (AB-8) Isolation[a]

components	sample amount (mg)	DPPH radical scavenging activity (%)[b]
ascorbic acid	1.0	93.1 ± 3.2
F10	2.0	8.6 ± 1.1
F30	2.0	55.2 ± 3.3
F50	2.0	41.5 ± 2.1
F70	2.0	9.4 ± 0.8
F90	2.0	6.3 ± 1.0

[a] The concentration of DPPH ethanolic–water solution was 65 μM. [b] Each value is the mean of triplicate measurements and the standard deviation (SD).

Biological Engineering, Zhejiang Gongshang University (Hangzhou, China). The apparatus (*10*) was equipped with a 1200 mL column with six-layer coils made of 5.0 mm i.d. Teflon tubing. For the separation, a K-1800 Wellchrom preparative high-performance liquid chromatography (HPLC) pump (Knauer, Germany), a 100 mL sample loop made of 3 mm i.d. Teflon tubing, and a B-684 collector (Büchi, Switzerland) with 15 mL tube racks was used. The suitable solvent systems were selected by partitioning experiments and evaluated by thin-layer chromatography (TLC) tests. For the separation of F30, the solvent system was composed of $CHCl_3$/MeOH/2-propanol/H_2O (5:6:1:4, v/v), the aqueous upper phase used as the stationary phase, and $CHCl_3$/MeOH/H_2O (13:7:8, v/v) was used for the separation of F50 also with the upper phase as the stationary phase. The sample solutions were prepared by dissolving 1.0 g of fraction F30 or F50 in 100 mL of the mobile phase, respectively.

The separation experiment for each sample was conducted with the solvent system described above. The coil column was first entirely filled with stationary phase. Then, the apparatus was rotated at 700 rpm and the sample solution was injected into the CCC system through the Teflon sample loop with the mobile phase at a flow rate of 3.0 mL/min. The effluent was collected into 15 mL tubes for TLC analysis. TLC analysis was performed on a GF_{254} (Merck) plate, using $CHCl_3$/MeOH/H_2O (7:3:1) as the developing solvent. The visualization was carried out with 10% sulfuric acid in ethanol as the spray reagent and heating on a hot plate at 110 °C.

Electrospray Ionization Mass Spectrometry (ESI-MS) and NMR. All ESI-MS experiments were performed on a Bruker Esquire LC-MS ion trap multiple mass spectrometer (Bremen, Germany) in positive and negative ionization mode analyzing ions up to *m*/*z* 2200. ^{1}H, ^{13}C, and DEPT 90/135 NMR spectra were recorded in CD_3OD on a Bruker Avance 500 (Karlsruhe, Germany) with 500 MHz for ^{1}H measurements and 125 MHz for ^{13}C measurements, respectively.

Evaluation of Antioxidant Activity. The radical scavenging activity was performed by the DPPH assay following Brand-Williams et al. (*11*) and Rotondi et al. (*12*) to evaluate the antioxidant activity of the purified components from sponge gourds. In our test protocol, 2.9 mL of 65 μM DPPH reagent in methanol/water (80:20, v/v) was added to 0.1 mL of 1.0% sample solution (1 mg/0.1 mL) to be tested. After 30 min of reaction at 25 °C, the absorbance was measured at $\lambda = 515$ nm and compared to a control sample prepared with 0.1 mL of methanol/water (80:20, v/v) solution without adding samples. The radical scavenging activity was expressed as percentage of DPPH radical elimination calculated according to the following equation:

$$\text{Rs}\ (\%) = (A_0 - A_1)/A_0 \times 100\%$$

where Rs is the radical scavenging activity of phenolic compounds, A_0 is the absorbance of sample at reaction time $t = 0$ min, and A_1 is the absorbance of sample at $t = 30$ min of the reaction. All tests were run in triplicate, and the average value was calculated.

HPLC Determination of Phenolic Compounds in Sponge Gourd. One gram of freeze-dried sample was powdered and extracted with 100 mL of 80% methanol for 1 h at 60 °C. The extract solution was evaporated in a vacuum at 40 °C to remove methanol. Then, the residual solution was partitioned twice with isovolumetric chloroform to remove lower polarity components and subsequently evaporated to remove the chloroform from the solution. Finally, the solution was concentrated to 20 mL for HPLC determination. The HPLC system was composed of an Alliance 2695, a 150 mm × 3.9 mm i.d., 5 μm Symmetry C-18 column, a 996 PDA detector, and a Millennium HPLC 2010 processing system (Waters, Milford, MA). A gradient elution was performed for the separation of fractions with a gradient 95% A to 85% A from 0 to 10 min and 85 to 40% A from 10 to 20 min, where A was 0.05% formic acid in water and the other part of the mobile phase was 0.05% formic acid in methanol.

Table 2. DPPH Radical Scavenging Rate of the Substances from HSCCC Isolations of F30 and F50[a]

isolated from	compounds	sample amount (mg)	DPPH radical scavenging activity (%)[b]
F30	ascorbic acid	1	93.1 ± 3.2
	component I	2	25.2 ± 1.3
	component II	2	31.5 ± 2.0
	component III	2	65.1 ± 2.1
	1	1	96.3 ± 2.7
	2	1	83.6 ± 2.9
	3	1	85.2 ± 1.7
	4	1	90.4 ± 3.8
	5	1	71.2 ± 1.5
F50	component IV	2	41.7 ± 3.3
	component V	2	33.4 ± 1.6
	6	1	77.3 ± 2.4
	7	1	76.6 ± 2.2
	8	1	81.2 ± 1.3

[a] The concentration of DPPH reagent in ethanol–water solution was 65 μM. [b] Each value is the mean of triplicate measurements and the SD.

RESULTS AND DISCUSSION

Antioxidant Activity of Fractions Recovered by Organic Gel Isolation (Macroporous Resin AB-8). The ethanolic extract from sponge gourds was separated by macroporous resin adsorption chromatography to yield five fractions: F10, F30, F50, F70, and F90. During our antioxidant activity-guided isolation of sponge gourd constituents, the experiments showed that solutions of fractions F30 and F50 with concentrations of 2 mg/0.1 mL showed the highest DPPH radical scavenging activity at a rate of 55.2 and 41.5%, respectively. In comparison, the standard antioxidant, ascorbic acid, showed almost double activity at half concentration (1 mg/0.1 mL) with a value of 93.1%. For the fractions F10, F70, and F90, a significant scavenging activity of DPPH radical was not determined (**Table 1**). Following these antioxidant results, further preparative isolations were done for fractions F30 and F50 to yield pure compounds **1**−**8**.

Antioxidant Activity of the Substances Isolated by HSCCC. HSCCC is an all-liquid chromatographic system, working without solid support, and separation is based on fast partitioning effects of the analytes between two immiscible liquid phases. Irreversible adsorbing effects and artifact formation is minimized. Application of HSCCC in natural product chemistry is steadily increasing, because of its superior separation abilities and excellent recovery rates. The above advantages are favorable for the recovery of phenolic compounds in plant extracts, which are easily adsorbed by solid support in conventional column chromatographic separation.

The HSCCC separation of 1 g of fraction F30 was performed with a biphasic solvent system composed of $CHCl_3$/MeOH/2-propanol/H_2O (5:6:1:4, v/v) using the aqueous upper phase as the stationary phase. TLC analysis of the chromatographic fractions showed that tubes 12−22, 30−36, 60−78, 82−106, 158−180, 194−240, and 250−310 consisted of five substances

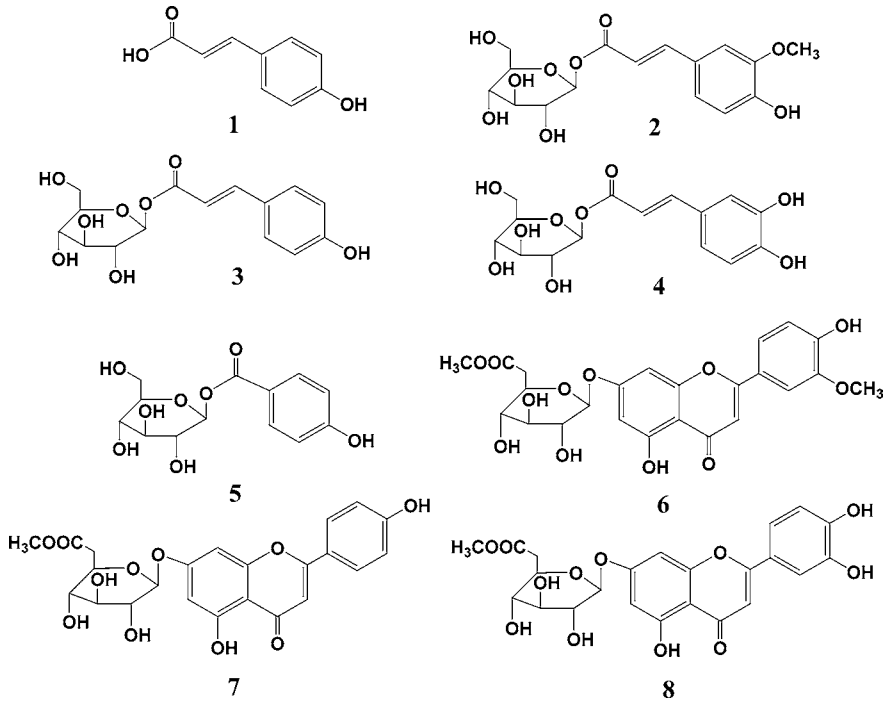

Figure 1. Antioxidant components isolated from the fruits of *L. cylindrica* (sponge gourd). *p*-Coumaric acid, **1**; 1-*O*-feruloyl-β-D-glucose, **2**; 1-*O*-*p*-coumaroyl-β-D-glucose, **3**; 1-*O*-caffeoyl-β-D-glucose, **4**; 1-*O*-(4-hydroxybenzoyl)glucose, **5**; diosmetin-7-*O*-β-D-glucuronide methyl ester, **6**; apigenin-7-*O*-β-D-glucuronide methyl ester, **7**; and luteolin-7-*O*-β-D-glucuronide methyl ester, **8**.

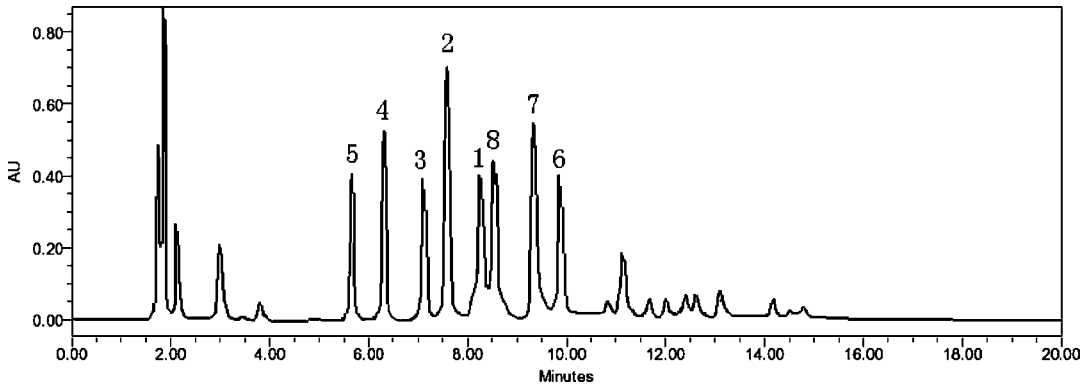

Figure 2. RP C-18 HPLC analysis ($\lambda = 254$ nm) of the compounds in the ethanolic extract of the fruits of *L. cylindrica* (sponge gourd). Compounds: **1**, *p*-coumaric acid; **2**, 1-*O*-feruloyl-β-D-glucose; **3**, 1-*O*-*p*-coumaroyl-β-D-glucose; **4**, 1-*O*-caffeoyl-β-D-glucose; **5**, 1-*O*-(4-hydroxybenzoyl)glucose; **6**, diosmetin-7-*O*-β-D-glucuronide methyl ester; **7**, apigenin-7-*O*-β-D-glucuronide methyl ester; and **8**, luteolin-7-*O*-β-D-glucuronide methyl ester.

and were combined and evaporated under vacuum to yield component I (0.31 g, tubes 30−36), component II (0.12 g, tubes 60−78), and five relatively pure substances. After the separation, the solvent in the coil column was pushed out and evaporated under vacuum to give 0.2 g of component III.

Before further analysis, the five substances were decolorized with active carbon to yield 23 mg of compound **1**, 70 mg of compound **2**, 36 mg of compound **3**, 43 mg of compound **4**, and 31 mg of compound **5** with purity more than 95% analyzed by HPLC, respectively.

The chromatographic separation of 1 g of F50 with the biphasic HSCCC solvent system composed of $CHCl_3$/MeOH/water (13:7:8, v/v) was carried out using the upper phase as the stationary phase. The separation resulted in the fractions 14−28, 36−64, 70−106, and 114−150, which displayed single spots on the TLC plate. The fractions were combined and evaporated in a vacuum to yield component IV (0.27 g, tubes 14−28) and three substances, which were decolorized with active carbon. Finally, 42 mg of compound **6**, 57 mg of compound **7**, and 33 mg of compound **8** were obtained. HPLC analysis showed more than 99% purity of the isolated substances. After the separation, the solvent in the coil column was pushed out and evaporated under vacuum to give 0.35 g of component V.

Table 2 shows the DPPH radical scavenging rate of components I−V and the compounds **1**−**8** from the HSCCC separation. In the following order, substances **1**−**8** gave the DPPH radical scavenging rates 96.3, 83.6, 85.2, 90.4, 71.2, 77.3, 76.6, and 81.2% while the activity of ascorbic acid was 93.1%. The recovered compounds **1**−**8** are water soluble phenolic compounds and have strong antioxidant activity in the DPPH radical scavenging assay. Components I−V also showed antioxidant activity with DPPH radical scavenging rates of 25.2, 31.5, 65.1, 41.7, and 33.4%, respectively. The constituents of components I−V require further isolation and identification to screen antioxidant substances.

Structure Elucidation of the Compounds Possessing Antioxidant Activity. Comparing the MS, ^{1}H NMR, and ^{13}C NMR with those of reported in the literature (*13*, *14*), we identified compounds **1**−**5** as *p*-coumaric acid (**1**), 1-*O*-feruloyl-β-D-

glucose (**2**), 1-*O*-*p*-coumaroyl-*β*-D-glucose (**3**), 1-*O*-caffeoyl-*β*-D-glucose (**4**), and 1-*O*-*β*-(4-hydroxybenzoyl)glucose (**5**). ^{1}H- and ^{13}C NMR resonances indicated that substances **6**−**8** possess a similar flavone skeleton and a glucuronide methyl ester moiety, and all MS, ^{1}H NMR, and ^{13}C NMR data are in accordance to the related compounds in the literature (*15*−*19*). Compounds **6**−**8** were unambiguously identified as diosmetin-7-*O*-*β*-D-glucuronide methyl ester (**6**), apigenin-7-*O*-*β*-D-glucuronide methyl ester (**7**), and luteolin-7-*O*-*β*-D-glucuronide methyl ester (**8**), respectively. Compounds **1**−**8** (**Figure 1**) were isolated from this plant for the first time.

In our DHHP radical scavenging assay performed on the eight compounds, the antioxidant activities at the same mass were in decreasing order **1** > ascorbic acid > **4** > **3** > **2** > **8** > **6** > **7** > **5**. The stronger activity of compound **1** without glycosyl than compound **3** indicates that the glycosyls do not contribute an effect on radical scavenging. Comparing to the activities of compounds **2**−**4** or **6**−**8**, we can find that the phenolic hydroxyl group on the 4-position of the benzene ring plays a key role on radical scavenging while the hydroxyl group on the 3-position also gives some contribution to radical scavenging. However, the methoxyl group on the 3-position seems to have no effect on radical scavenging. In principle, the structure−radical scavenging relationships of the phenolic compounds and flavonoids are in accordance to literature data (*20*−*23*).

The constituents in fruits and vegetables possessing antioxidant activity, for example, vitamin C, carotenoids, and the compounds containing phenolic hydroxyl groups such as catechins, phenylpropanoids, flavonoids, and anthocyanins, possess preventive effects on cardiovascular disease and cancer (*24*−*27*). The present study reveals that the compounds from the fruits of *L. cylindrica* such as cinnamic acid derivates (1-*O*-feruloyl-*β*-D-glucose, 1-*O*-*p*-coumaroyl-*β*-D-glucose, *p*-coumaric acid, and 1-*O*-caffeoyl-*β*-D-glucose) and the flavonoid glycosides (diosmetin-7-*O*-*β*-D-glucuronide methyl ester, apigenin-7-*O*-*β*-D-glucuronide methyl ester, and luteolin 7-*O*-*β*-D-glucuronide methyl ester) are phenolic substances of high antioxidant potential. The total amount of the eight compounds comprised 6.6% of the ethanolic extract, i.e., 0.66% of the dried fruits, calculated from the obtained amounts recovered by HSCCC. A determination of each compound (**Figure 2**) showed that the contents of compounds **1**−**8** were 0.05, 0.16, 0.08, 0.10, 0.07, 0.15, 0.21, and 0.12%, respectively, which gave a total content of 0.94% in dried skin-peeled gourd. The total amounts of phenolic compounds and flavonoids are consistent to those determined by spectrophotometer assays, reported by Bor et al. (*3*). A daily intake of fresh sponge gourd is about 200−500 g, which contains about 60−160 mg of antioxidant constituents, a total of compounds **1**−**8**. Therefore, the consumption of sponge gourds can supply some antioxidant constituents to the human body.

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LITERATURE CITED

(1) Borek, C. Antioxidant health effects of aged garlic extract. *J. Nutr.* **2001**, *131*, 1010−1015.

(2) Miller, N. J. Flavonoids and phenylpropanoids as contributors to the antioxidant activity of fruit juices. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Marcel Dekker: New York, 1998; p 387.

(3) Bor, J.-Y.; Chen, H.-Y.; Yen, G.-C. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *J. Agric. Food Chem*. **2006**, *54*, 1680−1686.

(4) Ansari, N. M.; Houlihan, L.; Hussain, B.; Pieroni, A. Antioxidant activity of five vegetables traditionally consumed by south-asian migrants in Bradford, Yorkshire, UK. *Phytother. Res.* **2005**, *19*, 907−911.

(5) Liang, L.; Liu, C. Y.; Li, G. Y.; Lu, L. E.; Cai, Y. C. Studies on the chemical components from leaves of *Luffa cylindrica* Roem. *Yaoxue Xuebao* **1996**, *31*, 122−125.

(6) Takemoto, T.; Arihara, S.; Yoshikawa, K.; Kusumoto, K.; Yano, I.; Hayashi, T. Studies on the constituents of Cucurbitaceae plants. VI. On the saponin constituents of *Luffa cylindrica* Roem. *Yakugaku Zasshi* **1984**, *104*, 246−255.

(7) Xiong, S.; Fang, Z.; Zeng, X. Studies on the chemical constituents of *Luffa cylindrica* (L.) Roem. *China J. Chin. Mater. Med.* **1994**, *19*, 233−234.

(8) Du, Q.; Chen, P.; Jerz, G.; Winterhalter, P. Preparative separation of flavonoid glycosides in leaves extract of *Ampelopsis grossedentata* using high-speed counter-current chromatography. *J. Chromatogr. A* **2004**, *1040*, 147−149.

(9) Du, Q.; Zhang, Q.; Ito, Y. Isolation and identification of phenolic compounds in the fruit of *Benincasa hispida* by HSCCC. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 137−144.

(10) Du, Q.; Li, Z.; Ito, Y. Preparative separation of isoflavone components in soybeans using high-speed countercurrent chromatography. *J. Chromatogr. A* **2001**, *923*, 271−274.

(11) Brand-Willams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Food Sci. Technol.* **1995**, *28*, 25−30.

(12) Rotondi, A.; Bendini, A.; Cerretani, L.; Mari, M.; Lercker, G.; Toschi, T. G. Effect of olive ripening degree on the oxidative stability and organoleptic properties of Cv. Nostrana di Brisighella extra virgin olive oil. *J. Agric. Food Chem*. **2004**, *52*, 3649−3654.

(13) Jiang, Z.; Hirose, Y.; Iwata, H.; Sakamoto, S.; Tanaka, T.; Kouno, I. Caffeoyl, coumaroyl, galloyl, and hexahydroxydiphenoyl glucoses from *Balanophora japonica*. *Chem. Pharm. Bull.* **2001**, *49*, 887−892.

(14) Kaneko, T.; Ohtani, K.; Kasai, R.; Yamasaki, K.; Nguyen, M. D. N-alkyl glycosides and *p*-hydroxybenzoyloxy glucose from fruits of *Crescentia cujete*. *Phytochemistry* **1998**, *47*, 259−263.

(15) Lee, M. H.; Son, Y. K.; Han, Y. N. Tissue factor inhibitory flavonoids from the fruits of *Chaenomeles sinensis*. *Arch. Pharm. Res.* **2002**, *25*, 842−850.

(16) Matsuda, H.; Cai, H.; Kubo, M.; Tosa, H.; Inuma, M. Study on anti-cataract drugs from natural sources. II. Effects of Buddlejae Flos on in vitro aldose reductase activity. *Biol. Pharm. Bull.* **1995**, *18*, 463−466.

(17) Dapkevicius, A.; Van Beek, T. A.; Lelyveld, G. P.; Van Veldhuizen, A.; De Groot, A.; Linssen, J. P. H.; Venskutonis, R. Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *J. Nat. Prod.* **2002**, *65*, 892−896.

(18) Schulz, M.; Strack, D.; Weissenböck, G.; Markham, K. R.; Dellamonica, G.; Chopin, J. Two luteolin glucuronides from primary leaves of *Secale cereale*. *Phytochemistry* **1985**, *24*, 343−345.

(19) Woo, E.; Piao, M. S. Antioxidative Constituents from *Lycopus lucidus*. *Arch. Pharm. Res.* **2004**, *27*, 173−176.

(20) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(21) Chen, J. H.; Ho, C.-T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* **1997**, *45*, 2374−2378.

(22) Sroka, Z.; Cisowski, W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food Chem. Toxicol.* **2003**, *41*, 753−758.

(23) Raneva, V.; Shimasaki, H.; Ishida, Y.; Ueta, N.; Niki, E. Antioxidative activity of 3,4-dihydroxyphenylacetic acid and caffeic acid in rat plasma. *Lipids* **2001**, *36*, 1111−1116.
(24) Rice-Evans, C. A.; Packer, L. *Flavonoids in Health and Disease*; Marcel Dekker: New York, 1998.
(25) Maxwell, S. R. J.; Lip, G. Y. H. Free radicals and antioxidants in cardiovascular disease. *Br. J. Clin. Pharmacol*. **1997**, *44*, 307−317.
(26) Hayes, J. D.; McMahon, M. Molecular basis for the contribution of the antioxidant responsive element to cancer chemoprevention. *Cancer Lett.* **2001**, *174*, 103−113.
(27) Wen, J.; You, K.-R.; Lee, S.-Y.; Song, C.-H.; Kim, D.-G. Oxidative stress-mediated apoptosis. The anticancer effect of the sesquiterpene lactone parthenolide. *J. Biol. Chem*. **2002**, *277*, 38954−38964.

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