JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of Six Phenylpropanoids from Garlic Skin as Major Antioxidants

MAKOTO ICHIKAWA,*,† KENJIRO RYU,† JIRO YOSHIDA,† NAGATOSHI IDE,† YUKIHIRO KODERA,† TAKASHI SASAOKA,† AND ROBERT T. ROSEN‡

Healthcare Research Institute, Wakunaga Pharmaceutical Company, Ltd., 1624 Shimokotachi, Kodacho, Takatagun, Hiroshima 739-1195, Japan, and Center for Advanced Food Technology, Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901-8520

The extract of garlic skins (peels) showed strong antioxidant activity, and some responsible constituents were isolated and identified. Garlic (*Allium sativum* L.) has been used as an herbal medicine, but there is no report on the health benefits of the skin or peel. In this study, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of garlic skin extract was evaluated. Using chromatographic techniques, the active constituents were isolated and subsequently identified. Analyses by high-performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA) suggested that these compounds were phenylpropanoids, which had a characteristic absorbance at 300−320 nm. Liquid chromatography−mass spectrometry (LC-MS) and nuclear magnetic resonance analyses allowed the chemical structures of the isolated constituents to be postulated. The proposed compounds were subsequently synthesized and compared with the constituents in the extract using HPLC-PDA and LC-MS. *N-trans*-Coumaroyloctopamine, *N-trans*-feruloyloctopamine, guaiacylglycerol-β-ferulic acid ether, and guaiacylglycerol-β-caffeic acid ether were identified as were *trans*-coumaric acid and *trans*-ferulic acid. Also, the antioxidant activities of these compounds were determined.

KEYWORDS: ***Allium sativum*; garlic; phenylpropanoid; antioxidant; *trans*-coumaric acid; *trans*-ferulic acid; *N-trans*-coumaroyloctopamine; *N-trans*-feruloyloctopamine; guaiacylglycerol-β-ferulic acid ether; guaiacylglycerol-β-caffeic acid ether**

INTRODUCTION

Garlic (*Alliium sativum* L.) has been used universally as a flavoring ingredient, functional food, and traditional medicine. Using modern scientific techniques, the biological effects of garlic have been studied for decades. There are a number of studies demonstrating that garlic can prevent or ameliorate various types of diseases such as cardiovascular disease, cancer, and age-related disease (*1*). In contrast, garlic skins or peels have not been studied for their health benefits because they are not an edible part of garlic. There are a few reports on the chemical composition of garlic skins. It has been reported that the characteristic constituent in garlic skins is pectin (*2*−*4*). Also, Schmidtlein et al. (*5*) reported that the enzymatic hydrolysate of garlic skins contained *p*-coumaric acid, ferulic acid, and sinapic acid. Onion skin extracts have been reported to have antioxidant activity and an inhibitory effect on xanthine oxidase (*6*−*8*). On the basis of these discoveries, some dietary supplements made of onion skins have been sold in the functional food and nutraceutical market in Japan, but there are no supplements available based on garlic skins.

In this study, we evaluated the antioxidant activities of garlic skin extract, which showed a strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect. Subsequently, we investigated the antioxidant constituents in garlic skins. Six phenolic compounds were isolated and identified using high-performance liquid chromatography with a photodiode array detector (HPLC-PDA), liquid chromatography−mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy. The antioxidant activities of these compounds were also determined.

MATERIALS AND METHODS

General. NMR experiments were performed on a JNM-ECP500 (JEOL, Tokyo, Japan) operating at 500 MHz for ^{1}H and 126 MHz for ^{13}C, respectively. Semipreparative HPLC was performed with a 600E HPLC pump (Waters, Milford, MA) coupled to a Spectro Monitor 3100 variable-wavelength detector (Milton Roy, Storrs, CT).

Reagents. Acetonitrile, ascorbic acid, DPPH, ethanol, ethyl acetate, formic acid, hexane, and methanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The solvents used in HPLC-

* Author to whom correspondence should be addressed (telephone +81-826-45-2331; fax +81-826-45-4351; e-mail ichikawa_m@wakunaga.co.jp).
† Wakunaga Pharmaceutical Co., Ltd.
‡ Rutgers, The State University of New Jersey.

10.1021/jf034791a CCC: $25.00
Published on Web 11/07/2003

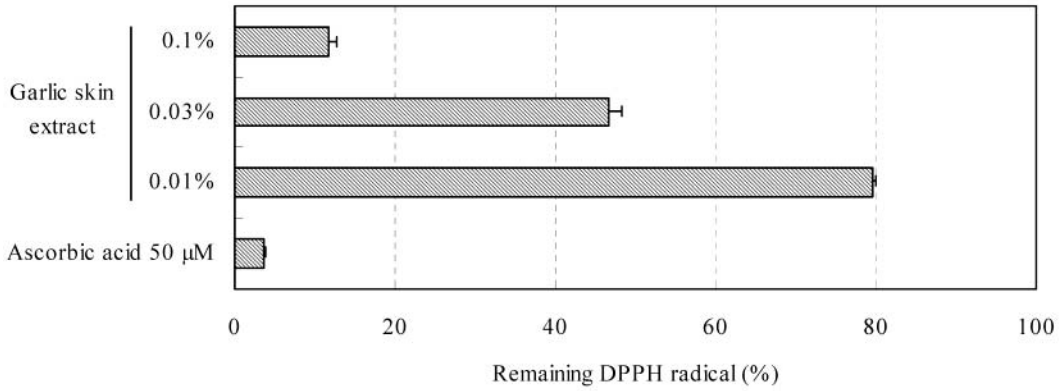


Figure 1. DPPH radical scavenging activity of 80% ethanol extract of garlic skin. Ascorbic acid was tested as a positive control. The data are the mean of triplicate measurements.

Table 1. ^{1}H and ^{13}C NMR Assignment for Guaiacylglycerol Ethers[a]

	guaiacylglycerol-β-caffeic acid ether (**2**)		guaiacylglycerol-β-ferulic acid ether (**5**)	
position	δ_C	δ_H	δ_C	δ_H
1	134.0		133.8	
2	111.5	7.01, d (1.8)	111.8	7.03, d (1.8)
3	149.0		148.9	
4	147.4		147.2	
5	116.0	6.74, d (8.3)	115.9	6.75, d (7.8)
6	120.7	6.84, dd (1.8, 8.3)	120.7	6.86, dd (2.3, 7.8)
7	74.0	4.92, d (5.5)	73.9	4.90, d (5.5)
8	86.7	4.28, m	86.3	4.44, m
9	61.9	3.54, dd (5.3, 11.7) 3.77, dd (4.4, 11.7)	62.0	3.51, dd (5.5, 11.9) 3.76, dd (3.7, 11.9)
1′	131.4		130.0	
2′	115.6	7.04, d (2.3)	112.3	7.22, d (1.8)
3′	149.4		151.7	
4′	149.5		151.8	
5′	118.0	6.97, d (8.3)	117.7	7.05, d (8.3)
6′	121.4	6.92, dd (1.8, 8.3)	123.4	7.10, dd (1.8, 8.7)
7′	143.3	7.38, d (16.0)	145.6	7.57, d (16.0)
8′	121.4	6.31, d (16.0)	118.3	6.37, d (16.0)
9′	172.6		171.4	
3-OMe	56.3	3.81, s	56.4	3.82, s
3′-OMe			56.7	3.90, s

[a] All data were obtained in CD_3OD with tetramethylsilane as the internal standard. Coupling constants (J in hertz) are given in parentheses. d, doublet; dd, double doublet; m, multiplet; s, singlet.

PDA and LC-MS analyses were of HPLC grade. *trans*-Coumaric acid (**1**) and *trans*-ferulic acid (**3**) were purchased from Aldrich Chemical Co., Inc. (St. Louis, MO).

Extraction and Isolation. One hundred grams of garlic skins was pulverized into small pieces by a model 7012 laboratory blender (Waring Products, Inc., Torrington, CT) and extracted with 4 L of 80% ethanol two times. The extract was pooled and concentrated under vacuum, and 4 g of 80% ethanol extract was obtained. Two grams of this extract was subjected to a column chromatography on 100 mL of MCI gel CHP-20P (Mitsubishi Chemical Corp., Tokyo, Japan) eluted with 25% methanol (200 mL), 50% methanol (200 mL), and 75% methanol (200 mL) followed by 100% methanol (500 mL). Each fraction was evaporated and dried under vacuum. The yields of 25, 50, 75, and 100% methanol eluates were 1245, 81, 147, and 257 mg, respectively. The 75% methanol eluate was subjected to semipreparative HPLC to isolate the antioxidant constituents in this fraction. The conditions were as follows: column, 250 × 10 mm Discovery C18 (Supelco, Bellefonte, PA); mobile phase, 0.05% formic acid/acetonitrile (85:15); flow rate, 3 mL/min; detection, 320 nm. Finally, four antioxidant constituents were isolated. To elucidate their chemical structure, the NMR spectra of these compounds were measured, and the assignments of each signal in the NMR spectra were conducted. The NMR data of guaiacylglycerol-β-caffeic acid ether (**2**) and guaiacylglycerol-β-ferulic acid ether (**5**) are summarized in **Table 1**.

HPLC-PDA Analysis. An LC-10AVP system coupled with an SPD-M10AVP photodiode array detector (Shimadzu, Kyoto, Japan) was used. The LC conditions were as follows: column, 150 × 2.0 mm TSK gel ODS-80Ts (Tosoh Corp., Tokyo, Japan); mobile phase, 0.1% formic acid/acetonitrile (85:15); flow rate, 0.2 mL/min; injection volume, 2 μL.

LC-MS Analysis. An 1100 system (Agilent, Palo Alto, CA) coupled with an LCQ mass spectrometer (ThermoFinnigan, San Jose, CA) was used. The LC conditions were the same as for the HPLC-PDA analysis. Mass spectra were acquired with an APCI probe in negative ion mode. The MS conditions were as follows: capillary voltage, 3 V; capillary temperature, 150 °C; vaporizer temperature, 450 °C.

DPPH Radical Scavenging Assay. Measurement of DPPH radical scavenging activity was performed according to a method previously reported (*9*) with some modification. Fifty microliters of sample solution was added into 950 μL of DPPH solution (100 μM in methanol). After incubation at 25 °C for 30 min, the absorbance at 515 nm was measured. The percentages of remaining DPPH radicals were calculated by comparing the absorbances between the sample and the control. Ascorbic acid was tested as a positive control.

Synthesis. *N-trans*-Coumaroyloctopamine (**4**) and *N-trans*-feruloyloctopamine (**6**) were synthesized as described by Negrel et al. (*10*) with some modifications. These synthesized compounds were refined using silica gel column chromatography. The conditions were as follows: column, 200 × 10 mm silica gel 60 (Merck, Darmstadt, Germany); eluate, 200 mL of ethyl acetate/hexane (1:1) containing 0.1% formic acid followed by 200 mL of ethyl acetate/hexane (4:1) containing 0.1% formic acid; fractions, 10 mL each. Each fraction was analyzed by thin-layer chromatography (TLC) on silica gel 60 F254 (Merck) developed with ethyl acetate/hexane (4:1) containing 0.1% formic acid and detected by irradiation of UV light at 254 nm. The fractions containing the amide were pooled, evaporated, and dried under vacuum.

RESULTS AND DISCUSSION

The DPPH radical scavenging effects of 80% ethanol extract of garlic skin are shown in **Figure 1**. The extract indicated strong activity eliminating ~90% of the DPPH radicals at the concentration of 0.1%. This is the first report of the antioxidant activity of garlic skins.

Further fractionation was performed on an MCI gel CHP-20P column. The DPPH scavenging activities of its fractions were determined. The activities at the concentrations of 0.01% of 25, 50, 75, and 100% methanol eluates were 0, 90, 72, and 66%, respectively. Although the 50% methanol eluate showed the strongest antioxidant activity, the isolation of its active constituents was unsuccessful because of the low yield and the complicated composition. However, the 75% methanol eluate showed a chromatogram with several well-separated peaks in

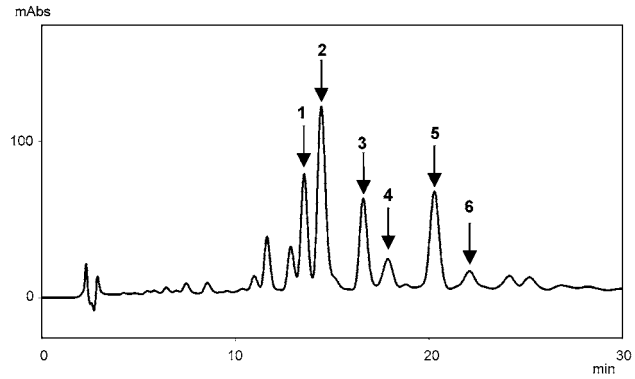


Figure 2. Chromatogram of the antioxidant fraction at 320 nm in HPLC-PDA analysis. Peaks 1–6 were suspected to be cinnamic acid derivatives.

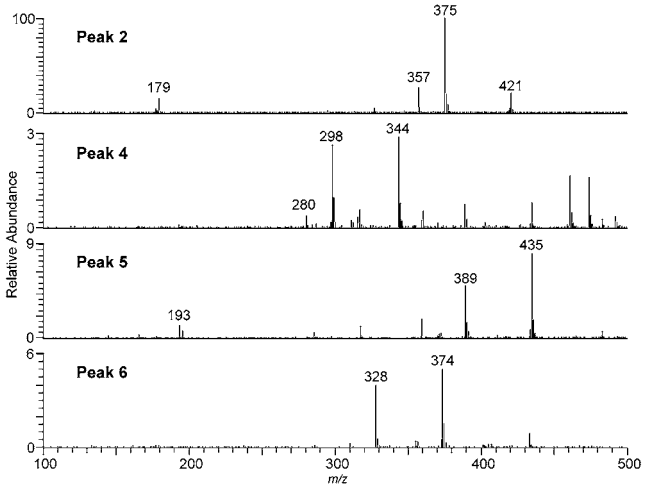


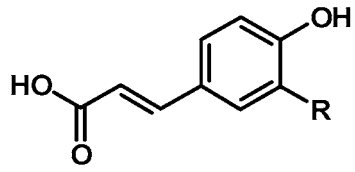
Figure 3. APCI negative ion mass spectra of peaks 2, 4, 5, and 6. Peak 2: *m/z* 421 = [M + formic acid − H]$^-$, 375 = [M − H]$^-$, 357 = [M − H_3O]$^-$, 179 = [M − guaiacylglycerol + OH]$^-$. Peak 4: *m/z* 344 = [M + formic acid − H]$^-$, 298 = [M − H]$^-$, 280 = [M − H_3O]$^-$; peaks more than *m/z* 350 were attributed to a coeluting impurity. Peak 5: *m/z* 435 = [M + formic acid − H]$^-$, 389 = [M − H]$^-$, 193 = [M − guaiacylglycerol + OH]$^-$. Peak 6: *m/z* 374 = [M + formic acid − H]$^-$, 328 = [M − H]$^-$.

the HPLC-PDA analysis as shown in **Figure 2**. We tried to isolate the active constituents from this fraction.

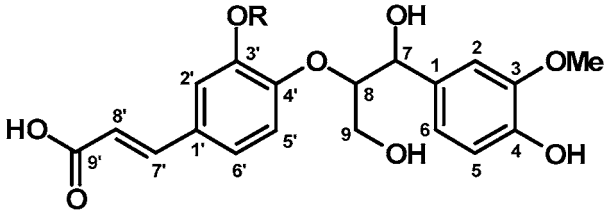
A characteristic absorbance at 300−320 nm in the UV spectra of peaks 1−6 in **Figure 2** suggested that these constituents were cinnamic acid derivatives. Several commercially available compounds were analyzed under the same HPLC conditions. Peaks 1 and 3 were identified as *trans*-coumaric acid (**1**) and *trans*-ferulic acid (**3**), respectively, because their retention times and UV spectra were identical with those of commercially purchased standards.

For further structure elucidation of the other peaks, LC-MS analyses were conducted. The mass spectra of peaks 2, 4, 5, and 6 are shown in **Figure 3**.

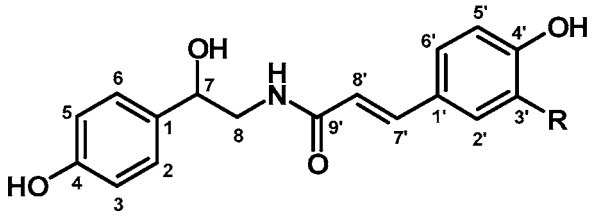
Peaks 2 and 5 were isolated and purified further using preparative HPLC. The mass spectra of peak 5 showed ions at *m/z* 389 [M − H]$^-$ and 435 [M + formic acid − H]$^-$ in the negative ion mode. The molecular weight of peak 5 is 390. The ^{1}H and ^{13}C NMR spectra of this compound were also determined. These indicated that this compound had two methoxyl groups, two aromatic rings, and one α,β-unsaturated carboxylic acid moiety. With the structural information obtained from the coupling constants, peak 5 was supposed to have a guaiacylglycerol moiety and a ferulic acid moiety. The signal assignments of NMR data were confirmed by the 2D NMR experiments including H−H COSY, HMQC, and HMBC. Because a correlation between the proton at 8 and the carbon at 4′ was observed in the HMBC experiment, it was supposed that the ferulic acid moiety was bound to guaiacylglycerol on the β-position. Finally, peak 5 was identified as guaiacylglycerol-β-ferulic acid ether (**5**). In the HPLC-PDA analysis, peak 2 showed a UV spectrum similar to that of peak 5. Also, the mass spectrum of peak 2 indicated the pseudomolecular ion peak at *m/z* 375 [M − H] $^-$ and 421, as the deprotonated molecular ion and its formic acid adduct ion, respectively. In addition, peaks 2 and 5 showed a fragmentation pattern with the observation of [M − guaiacylglycerol + OH]$^-$ ion peaks at *m/z* 179 and 193, respectively. In the ^{1}H NMR spectrum of peak 2, the signal of only one methoxyl group was observed. From the data described above, peak 2 was identified as guaiacylglycerol-β-caffeic acid ether (**2**).



trans-Coumaric acid (**1**): R=H
trans-Ferulic acid (**3**): R=OCH_3



Guaiacylglycerol-β-caffeic acid ether (**2**): R=H
Guaiacylglycerol-β-ferulic acid ether (**5**): R=CH_3



N-trans-Coumaroyloctopamine (**4**): R=H
N-trans-Feruloyloctopamine (**6**): R=OCH_3

Figure 4. Chemical structures of the compounds identified from garlic skin.

The mass spectra of peaks 4 and 6 indicated these constituents had odd molecular weights (*m/z* 298 [M − H]$^-$ and 328 [M − H]$^-$) in the negative ion mode, respectively, indicating that these contain at least one nitrogen atom in each molecule. Using preparative HPLC, peaks 4 and 6 were isolated and their ^{1}H NMR spectra were measured. The spectrum of peak 4 showed the signals of eight aromatic, two olefinic, and three other protons. Also, the coupling constants and 2D NMR data gave some information about the partial structure of this compound. With this spectroscopic information, peak 4 was determined to be *N-trans*-coumaroyloctopamine (**4**). The ^{1}H NMR spectrum of peak 6 was quite similar to that of compound **4** and showed the signals of seven aromatic and three methoxy protons, suggesting that this compound could be *N-trans*-feruloyloctopamine

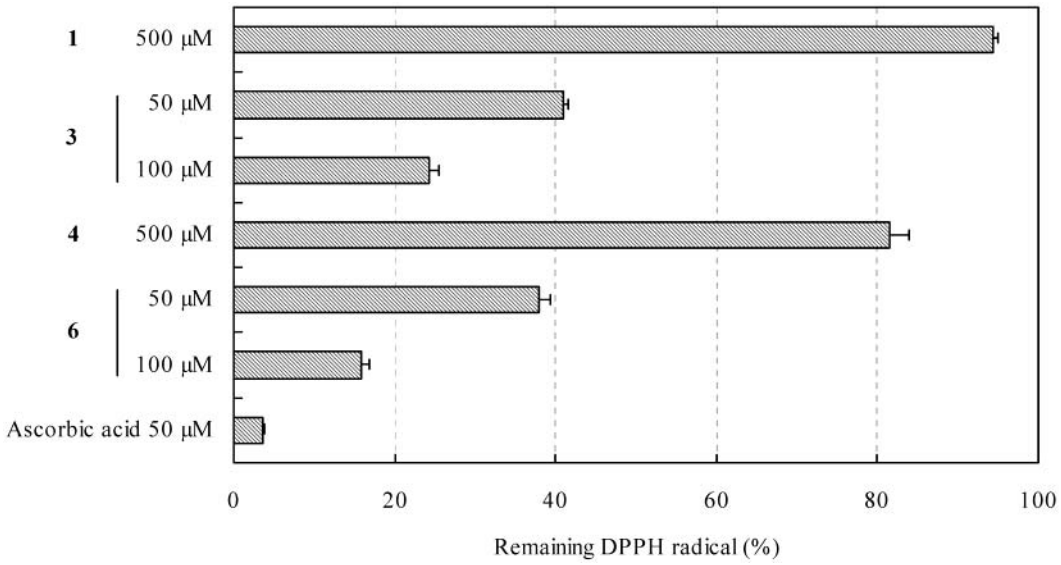


Figure 5. DPPH radical scavenging activity of *trans*-coumaric acid (**1**), *trans*-ferulic acid (**3**), *N-trans*-coumaroyloctopamine (**4**), and *N-trans*-feruloyloctopamine (**6**). Ascorbic acid was tested as a positive control. The data are the mean of triplicate measurements.

(**6**), compound **4** with one more methoxyl group. To confirm the structure elucidation of peaks 4 and 6, these suspected compounds were synthesized according to the method previously reported (*10*). The retention time and the mass spectra of the synthesized standards corresponded completely with those of the components observed during the LC-MS analysis, and the ^{1}H NMR data were also identical. The chemical structures of the identified compounds are shown in **Figure 4**. In the results of the HPLC analysis, the contents of compounds **1**, **2**, **3**, **4**, **5**, and **6** in the garlic skin were 6.5, 20.0, 5.9, 1.9, 13.7, and 1.5 μg/g, respectively.

The DPPH radical scavenging effects of some of these constituents were determined. Because compounds **2** and **5** were yielded in very small quantities, difficult to synthesize, and commercially unavailable, it was impossible to evaluate the antioxidant activities of these compounds in this study. The activities of compounds **1**, **3**, **4**, and **6** are shown in **Figure 5**. Compounds **3** and **6** showed strong DPPH radical scavenging effects. On the other hand, compounds **1** and **4** showed moderate activities. These results suggest that the methoxyl groups in compounds **3** and **6** are very critical for the radical scavenging effects. The methoxyl groups on the aromatic ring increase the stability of the phenoxy radicals. The fact that the DPPH radical scavenging effect was dependent on the number of hydroxyl (or methoxyl) groups on the benzene ring is consistent with the results reported by Kikuzaki et al. (*11*). In addition, the octopamine amides of cinnamic acid derivatives **4** and **6** showed slightly stronger activities than the corresponding free acid. As Son and Lewis (*12*) have reported, the presence of an additional proton-donating group (amide) might accelerate the radical scavenging activity. The antioxidant activities of these compounds were not strong enough to account for the activity of whole garlic skin. Other active constituents might be present in the extract and are under investigation.

This is the first report of guaiacylglycerol-β-caffeic acid ether (**2**) from a plant product. Compound **5** has been reported as a constituent of grass straws (*13*). Also, octopamine amides **4** and **6** have been reported as constituents of the root of eggplant and bell pepper (*14*, *15*). These amides were also produced as the early response of potato tubers against fungal attack and wounding (*10*).

In summary, we found that garlic skin extract had a strong DPPH radical scavenging activity, and six phenylpropanoid derivatives were identified as the primary antioxidant constituents from the extract. This is the first report of the possible health benefit of garlic skin. Presently, garlic skin is an industrial waste. The potential use of garlic skin in nutraceutical products may be an economical advantage for the industry.

ACKNOWLEDGMENT

We gratefully acknowledge Dr. Chi-Tang Ho and Dr. Shengmin Sang (Rutgers University) for helpful suggestions. We thank Dr. Elaine Fukuda and Joseph Lech (Rutgers University) for technical assistance.

LITERATURE CITED

(1) Rahman, K. Garlic and aging: New insights into an old remedy. *Ageing Res. Rev.* **2003**, *2*, 39−56.

(2) Abdel-Fattah, A. F.; Khaireldin, A. A. Pectin of garlic skins. *U. A. R. J. Chem.* **1970**, *13*, 27−37.

(3) Abdel-Fattah, A. F.; Edrees, M. A study on the composition of garlic skins and the structural features of the isolated pectic acid. *J. Sci. Food Agric.* **1972**, *23*, 871−877.

(4) Alexander, M. M.; Sulebele, G. A. Pectic substances in onion and garlic skins. *J. Sci. Food Agric.* **1973**, *24*, 611−615.

(5) Schmidtlein, H.; Herrmann, K. On the phenolic acids of vegetables. IV. Hydroxycinnamic acids and hydroxybenzoic acids of vegetables and potatoes. *Z. Lebensm. Unters.-Forsch.* **1975**, *159*, 255−263.

(6) Ra, K. S.; Suh, H. J.; Chung, S. H.; Son, J. Y. Antioxidant activity of solvent extract from onion skin. *Korean J. Food Sci. Technol.* **1997**, *29*, 595−600.

(7) Ra, K. S.; Bae, S. H.; Son, H. S.; Chung, S. H.; Suh, H. J. Inhibition of xanthine oxidase by flavonols from onion skin. *J. Korean Soc. Food Sci. Nutr.* **1998**, *27*, 693−697.

(8) Suh, H. J.; Lee J. M.; Cho, J. S.; Kim, Y. S.; Chung S. H. Radical scavenging compounds in onion skin. *Food Res. Int.* **1999**, *32*, 659−664.

(9) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.

(10) Negrel, J.; Javelle, F.; Paynot, M. Wound-induced tyramine hydroxycinnamoyl transferase in potato (*Solanum tuberosum*) tuber discs. *J. Plant Physiol.* **1993**, *142*, 518−524.

(11) Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **2002**, *50*, 2161−2168.

(12) Son, S.; Lewis, B. A. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: Structure−activity relationship. *J. Agric. Food Chem.* **2002**, *50*, 468−472.

(13) Jacquet, G.; Pollet, B.; Lapierre, C.; Mhamdi, F.; Rolando, C. New ether-linked ferulic acid-coniferyl alcohol dimers identified in grass straws. *J. Agric. Food Chem.* **1995**, *43*, 2746−2751.

(14) Yoshihara, T.; Takamatsu, S.; Sakamura, S. Three new phenolic amides from the roots of eggplant (*Solanum melongena* L.). *Agric. Biol. Chem.* **1978**, *42*, 623−627.

(15) Yoshihara, T.; Yamaguchi, K.; Takamatsu, S.; Sakamura, S. A new lignan amide, grossamide, from bell pepper (*Capsicum annuum* var. *grossum*). *Agric. Biol. Chem.* **1981**, *45*, 2593−2598.

Received for review July 16, 2003. Revised manuscript received September 25, 2003. Accepted October 2, 2003.

JF034791A