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Antioxidant Constituents of Radish Sprout (Kaiware-daikon), *Raphanus sativus* L.

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Methanol extracts of 11 kinds of commonly available vegetables were examined for hydroxyl radical scavenging potency using the bleomycin−Fe method. In this method, the iron ion and bleomycin in water form hydroxyl radicals, and the scavenging activity is monitored by the modified thiobarbituric acid method. All extracts showed scavenging capacity, even though the activity of some of them was lower than that of L-ascorbic acid. Those vegetables were classified into three groups according to their activity, groups showing strong activity, moderate activity, and weak activity, as compared to the activity of L-ascorbic acid at the same concentration. Among them, the methanol extract of radish sprout (Japanese name "kaiware-daikon") exhibited the highest potency (1.8 times as L-ascorbic acid). Then, we investigated the constituents of the methanol extract of radish sprout and the contribution to the overall activity of each compound by examining their activity. As the result, several kinds of sinapinic acid esters and flavonoids were isolated with high radical scavenging potency, which must contribute substantially to the activity.

KEYWORDS: Vegetable; antioxidation; *Raphanus sativus* L.; bleomycin; sinapate; flavonoid glycoside; hydroxyl radical

INTRODUCTION

It is often heard that we should consume several hundred grams of vegetables a day for our health. Of course, the reason may be due to the nutrients and dietary fibers that are rich in vegetables. However, vegetables belong to a very small category in the plant kingdom. The reason that these plants are regarded as "vegetables" has seldom been considered. The answer may be due not only to the abundance of nutrients and the richness in taste but also to some biological action on our health. The knowledge that vegetables act effectively for our health might have been accumulated by the experience of our ancestors during the long history of mankind, although nutritional studies of vegetables were only carried out during these past several decades. We always eat whole vegetables but not some limited compounds. L-Ascorbic acid, α-tocopherol, flavonoids, carotenoids, and polyphenols are the good examples from plants that are effective for our health. However, we hardly take them selectively from foods. Therefore, it is worthwhile to carry out a pharmaceutical evaluation using whole vegetables and their extracts so as to know effective ways to ingest them. In this study, we are interested in the pharmacological activities of vegetables, i.e., the differences in action, potency, and constituents, which contribute to the activity between vegetables. At the beginning, to get such information, we tried to compare the antioxidant activities of methanol extracts of 11 kinds of vegetables. Free radicals and active oxygens are thought to be the causative trigger of various diseases such as carcinogenesis, mutagenesis, diabetes mellitus, and arteriosclerosis (*1*). Recently, we found many reports on the antioxidant activity of vegetables or antioxidant active compounds in vegetables. Some studies investigated the antioxidant activity of the aqueous extract (*2*, *3*), while some examined specified constituents, such as phenolic compounds, in vegetables (*4*, *5*), and few reports gave us information on the antioxidant activity of whole extracts and compounds (*6*). So, we began an investigation of the relationships between the antioxidant activity and the constituents of each vegetable.

Moreover, there are many methods known to evaluate antioxidant (radical scavenging) activity. The mechanisms of these methods are based on the lipid peroxidation, scavenging radical ($OH^{\cdot}$, $O_2^{\cdot -}$, $HO_2^{\cdot}$, stable radicals such as DPPH, etc.), inhibition of oxidative enzyme, and so on. Even if a sample exhibits high activity with one of these methods, it does not always show similar good results with all other methods. Accordingly, we should evaluate samples accurately with several methods. This time, as the first screening to find out the difference in antioxidant potency of the methanol extract of vegetables, we examined antioxidant activity according to the bleomycin (BLM)−Fe method reported by Umezawa et al. (*7*). The mechanism of this method is described as shown in **Figure 1**. An aqueous solution of BLM in the presence of ferrous ion generates a hydroxyl radical. The radical and oxygen oxidize arachidonic acid to give malonaldehyde, and the product reacts

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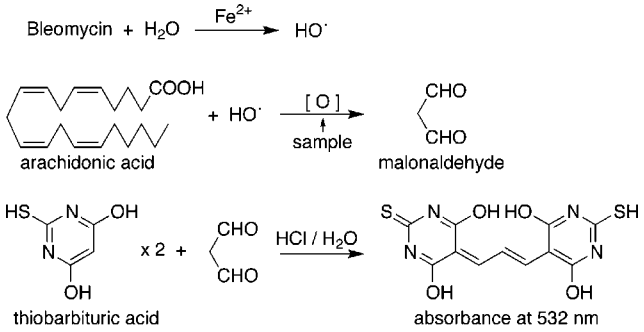

Figure 1. Mechanism of the BLM–Fe method.

with two molecules of 2-thiobarbituric acid (TBA) to form a chromophore possessing λ_{max} at 532 nm. This method takes a short time to give results with high reproducibility, although BLM is expensive. The hydroxyl radical is one of the most highly reactive radicals, which readily damages crucial substances in living organisms, such as DNA, carbohydrates, lipids, and proteins (*8*). Accordingly, it is of great interest for us to reduce the risk caused by such a radical, and the natural antioxidants in vegetables ingested as food must play an important role in overcoming such a risk.

With this method, we investigated the methanol extracts of 11 kinds of vegetables and classified them into three groups according to their antioxidant activity relative to that of L-ascorbic acid. On the basis of the above results, we further investigated the structures of the antioxidant constituents in *Raphanus sativus* L. (radish sprout), which showed the highest activity among the vegetables examined. We report herein the antioxidant acitivity of the methanol extract of vegetables and the structure and the respective activity of the constituents in radish sprout.

MATERIALS AND METHODS

Materials. All vegetables, *R. sativus* L. (radish sprout; Japanese name, kaiware-daikon; Brassicaceae), *Brassica oleracea* L. (cabbage, Brassicaceae), *Brassica rapa* L. var. *chinensis* (pak-choi, Brassicaceae), *Brassica rapa* L. var. *pervidis* B. (komatsuna, Brassicaceae), *Capsicum annuum* L. (sweet pepper, Solanaceae), *Spinacia oleracea* L. (spinach, Chenopodiaceae), *Cryptotaenia japanica* Hassk. (Japanese hornwort, Umbelliferae), *Chrysanthemum coronarium* L. (garland chrysanthemum, Compositae), *Asparagus officinalis* L. (asparagus, Liliaceae), *Allium sativum* L. (garlic sprout, Liliaceae), and *Allium fistulosum* L. (welsh onion, Liliaceae), were purchased at a market in Nagoya City. Arachidonic acid sodium salt, BLM sulfate (from *Streptomyces verticillus*) and TBA were all purchased from Sigma-Aldrich Co. Silica gel (BW-820MH, Fuji Silysia, Nagoya) and a thin-layer chromatography (TLC) plate (Kieselgel 60 F_{254} 5715, Merck) were used for column chromatography and analytical TLC, respectively. Medium-pressure column chromatography (MPLC) was performed using silica gel (60K230, Katayama Chemical Co. Ltd., Nagoya) and ODS (Develosil ODS 30/50, Nomura Chemical Co. Ltd.). The C-8 column for high-performance liquid chromatography (HPLC) was Develosil C8 (ϕ20 × 250, Nomura Chemical Co. Ltd.).

Antioxidant Assay. The antioxidant assay was carried out by the modified BLM−Fe(III) method reported by Umezawa et al. (*7*). Tris-HCl buffer (0.2 M; pH 7.4), 8 mM arachidonic acid, sample solution, 1 mM BLM, and 1.08 mM $FeSO_4$ (each 100 μL) were successively added to a test tube. After the solution was incubated at 37 °C for 5 min, to this solution was added 0.2 M HCl (10 μL) followed by 0.5% TBA (0.2 mL). This mixture was incubated at 37 °C for 30 min. Then, 0.4 mL of water and 1 mL of 1-butanol was added and the mixture was vigorously shaken and centrifuged at 300*g* for 10 min. Out of the resultant 1-butanol layer, 0.7 mL of the solution was diluted with 5.0 mL of 1-butanol in another tube. The absorbance of the solution at 532 nm (A_S) was measured by a spectrophotometer. Absorbance using water instead of a sample solution in the above protocol (A_C) served as the control. The antioxidant activity of the sample (AA_S) was expressed as the inhibition rate of the sample using the following formula.

$$\text{antioxidant activity } (AA_S) = (1 - A_S/A_C) \times 100\ (\%)$$

The concentration of sample solutions as well as the positive control, L-ascorbic acid, was fixed as 3 mg/mL for crude samples including the methanol extract and 1 mM for isolated compounds. At these concentrations, the antioxidant activity is in proportion to the concentrations of L-ascorbic acid, so as to get reliable data.

Index of Activity and Total Activity. The index of activity (I_A) and total activity (AA_T) were defined as follows, where AA_{VC} is the antioxidant activity of L-ascorbic acid at the same concentration of the samples, and the index of activity was expressed with a unit "VC", which means the vitamin C equivalent antioxidant activity.

$$\text{index of activity } (I_A) = A_S/A_{VC}\ (\text{VC})$$

$$\text{total activity } (AA_T) = I_A \times \text{content (\%) in the methanol extract}/100$$

Extraction of Vegetables for Antioxidant Assay. Each vegetable was completely soaked in methanol without homogenization or cut into small pieces and extracted three times at room temperature. This procedure was done within 7 days. The resultant methanol solution was evaporated in vacuo at under 35 °C to give the methanol extract. The methanol extract was subjected to the antioxidant assay.

Extraction and Partition of *R. sativus*. Fresh sprouts of *R. sativus* (4.3 kg) purchased at a market were brought to our laboratory and soon extracted three times with 10 L of methanol at room temperature within 7 days. The methanol extract (42.4 g), which was obtained by evaporation, was partitioned between water and hexane, ethyl acetate, and 1-butanol to give the corresponding solubles: hexane solubles (0.7 g, 1.65%), ethyl acetate solubles (3.9 g, 9.20%), 1-butanol solubles (13.0 g, 30.7%), and water solubles (24.0 g, 56.6%).

Fractionation of Hexane Solubles. Hexane solubles (0.7 g) were separated into two fractions by SiO_2 column chromatography using a mixed solvent of hexanes−ethyl acetate (5:1) to give RS-H1 (544 mg) and RS-H2 (135 mg). RS-H1 was further fractionated with an SiO_2 column using a mixed solvent of hexanes−ethyl acetate to afford methyl linolenate (**1**, 274 mg) (*9*), linolenic acid (**2**, 131 mg) (*9*), and a fraction (RS-H1-2). From RS-H1-2, phytol (**3**, 11.6 mg) (*10*) was obtained by preparative TLC using a mixed solvent of chloroform−acetone (20:1). RS-H2 was fractionated by MPLC equipped with an SiO_2 column using a mixture of benzene−acetone (20:1) as the eluent followed by SiO_2 column chromatography using the same solvent to give methyl sinapate (**4**, 13 mg) (*11*).

Methyl Linolenate (1). Yellow oil. IR (KBr): ν_{max} 1730 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.33 (6H, m, H-9, H-10, H-12, H-13, H-15, and H-16), 3.64 (3H, s, OMe), 2.78 (4H, m, H-8 and H-17), 2.27 (4H, t, J = 7.3 Hz, H-11 and H-14), 2.05 (2H, t, J = 7.4 Hz, H-2), 1.57 (2H, m, H-3), 1.28 (8H, m, H-4 − H-7), 0.95 (3H, t, J = 7.7 Hz, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.2 (s, C-1), 127.1, 127.7, 128.20, 128.23, 130.2, 131.9 (each d, C-9, C-10, C-12, C-13, C-15, and C-16), 51.4 (q, OMe), 34.0 (t, C-2), 29.05, 29.07, 29.11, 29.52 (each t, C-4 − C-7), 25.5, 25.6 (each d, C-11 and C-14), 24.8 (t, C-3), 20.5, 27.2 (each d, C-8 and C-17), 14.2 (q, C-18). FABMS *m*/*z* 293 $[M + H]^+$.

Linolenic Acid (2). Yellow oil. IR (KBr): ν_{max} 3584, 1713 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.35 (6H, m, H-9, H-10, H-12, H-13, H-15, and H-16), 2.79 (4H, m, H-8 and H-17), 2.30 (4H, t, J = 7.3 Hz, H-11 and H-14), 2.10 (2H, t, J = 7.3 Hz, H-2), 1.58 (2H, m, H-3), 1.28 (8H, m, H-4 − H-7), 0.94 (3H, t, J = 7.4 Hz, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 180.0 (s, C-1), 127.7, 127.8, 128.0, 128.2, 130.2, 131.9 (each d, C-9, C-10, C-12, C-13, C-15, and C-16), 34.0 (t, C-2), 29.01, 29.05, 29.11, 29.57 (each t, C-4 − C-7), 25.5, 25.6 (each d, C-11 and C-14), 24.6 (t, C-3), 20.5, 27.1 (each d, C-8 and C-17), 14.0 (q, C-18). FABMS *m*/*z* 279 $[M + H]^+$.

Phytol (3). Yellow oil. IR (KBr): ν_{max} 3375 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.39 (1H, t, J = 6.7 Hz, H-2), 4.13 (2H, d, J = 6.7 Hz, H-1), 2.00 (2H, t, J = 6.1 Hz, H-4), 1.64 (3H, s, 3-Me), 1.50 (1H, m, H-15), 1.05−1.42 (18H, m, H-5 − H-14), 0.84 (6H, d, J = 6.5 Hz, 16-Me and 17-Me), 0.82 (6H, d, J = 6.5 Hz, 7-Me and 11-Me). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 140.3 (s, C-3), 123.0 (d, C-2), 59.4 (t, C-1), 39.9 (t, C-4), 39.4 (t, C-14), 37.4 (t, C-8), 37.4 (t, C-12), 37.3 (t, C-10), 36.6 (t, C-6), 32.8 (d, C-7), 32.7 (d, C-11), 28.0 (d, C-15), 25.1 (t, C-5), 24.7 (t, C-13), 24.4 (t, C-9), 22.8 (q, C-17), 22.7 (q, C-16), 19.7 (2C, q, 7-Me and 11-Me), 16.2 (q, 3-Me). FABMS m/z 297 $[M + H]^+$.

Methyl Sinapate (4). Pale yellow amorphous solid. IR (KBr): ν_{max} 3420, 1670 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.58 (1H, d, J = 16.8 Hz, H-7), 6.90 (2H, s, H-2,6), 6.42 (1H, d, J = 16.8 Hz, H-8), 3.85 (6H, s, 3,5-OMe), 3.77 (3H, s, 9-OMe). ^{13}C NMR (methanol-d_4, 100 MHz): δ 168.6 (s, C-9), 149.2 (2C, s, C-3,5), 147.5 (d, C-7), 139.5 (s, C-4), 126.4 (s, C-1), 115.8 (d, C-8), 107.0 (2C, d, C-2,6), 56.8 (2C, q, 3,5-OMe), 52.1 (q, 9-OMe). FABMS m/z 239 $[M + H]^+$.

Fractionation of Ethyl Acetate Solubles. Ethyl acetate solubles (3.9 g) were fractionated into two fractions (RS-E1 and -E2) by SiO_2 column chromatography using a mixed solvent of chloroform−EtOAc (20:1). RS-E1 (664 mg) was fractionated with an SiO_2 column using a mixed solvent of benzene−acetone (40:1) to give **4** (22 mg) (*11*). RS-E2 (1.7 g) was further fractionated into three fractions (RS-E2-1−3) by SiO_2 column chromatography using a mixed solvent of chloroform−methanol (20:1). RS-E2-3 was separated with an SiO_2 column with the same solvent into six fractions (RS-E2-3-1−6). RS-E2-3-2 was 1,2-disinapoyl-β-D-glucopyranoside (**5**, 100 mg) (*12*). RS-E2-3-3 and RS-E2-3-5 were purified by MPLC using SiO_2 and a mixture of chloroform−methanol (20:1) as solvent to afford β-D-(3,4-dipinapoyl)-frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (**6**, 132 mg) (*13*) and 1-*O*-(6″,9″,12″15″-octadecateraenoyl)-3-*O*-β-D-galactopyranosyl glycerol (**7**, 102 mg) (*14*,*15*), respectively.

1,2-Disinapoyl-β-D-Glucopyranoside (5). Pale yellow amorphous solid; $[\alpha]_D$ −23.8° (*c* 1.0, MeOH). UV (MeOH): λ_{max} 331 (3.4), 240 (3.3), 225 (2.3). IR (KBr): ν_{max} 3444, 1633 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.63 (1H, d, J = 16.0 Hz, H-7′), 7.62 (1H, d, J = 15.9 Hz, H-7″), 6.87 (2H, s, H-2″,6″), 6.83 (2H, s, H-2′,6′), 6.39 (1H, d, J = 15.9 Hz, H-8″), 6.31 (1H, d, J = 16.0 Hz, H-8′), 5.79 (1H, d, J = 8.3 Hz, H-1), 5.06 (1H, t, J = 8.3 Hz, H-2), 3.87 (1H, br d, J = 12.0 Hz, H-6), 3.84 (3H, s, 3″,5″-OMe), 3.82 (3H, s, 3′,5′-OMe), 3.74 (1H, dd, J = 4.8, 12.0 Hz, H-6), 3.72 (1H, t, J = 8.3 Hz, H-3), 3.51 (1H, t, J = 8.3 Hz, H-4), 3.50 (1H, m, H-5). ^{13}C NMR (methanol-d_4, 100 MHz): δ 168.3 (s, C-9″), 167.1 (s, C-9′), 150.0 (2C, s, C-3′,5′ and C-3″,5″), 149.0 (d, 7′), 147.9 (d, C− 7″), 140.1 (s, C-4″), 139.7 (s, C-4′), 126.5 (s, C-1″), 126.3 (s, C-1′), 115.5 (d, C-8″), 114.5 (d, C-8′), 107.2 (d, C-2″,6″), 106.9 (sd, C-2′,6′), 94.0 (d, C-1), 79.1 (d, C-5), 76.0 (d, C-3), 74.3 (d, C-2), 71.3 (d, C-4), 62.3 (t, C-6), 56.8 (3C, q, 3′,5′-OMe and 3″,5″-OMe). HR-FABMS m/z 615.1684 ($[M + Na]^+$, 615.1633 calcd for $C_{28}H_{32}O_{14}Na$).

β-D-(3,4-Dipinapoyl)frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (6). Pale yellow amorphous solid; $[\alpha]_D$ −19.7° (*c* 0.9, MeOH). UV (MeOH): λ_{max} 329(3.6), 239, (3.5), 225 (3.5). IR (KBr): ν_{max} 3446, 1701, 1633 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.70 (1H, d, J = 15.8 Hz, H-7″), 7.55 (1H, d, J = 15.9 Hz, H-7‴), 7.46 (1H, d, J = 15.8 Hz, H-7⁗), 6.91 (2H, s, H-2″,6″), 6.81 (2H, s, H-2‴,6‴), 6.77 (2H, s, H-2⁗,6⁗), 6.48 (1H, d, J = 15.9 Hz, H-8‴), 6.45 (1H, d, J = 15.8 Hz, H-8″), 6.25 (1H, d, J = 15.8 Hz, H-8⁗), 5.79 (1H, d, J = 7.5 Hz, H-3), 5.55 (1H, d, J = 3.9 Hz, H-1′), 5.06 (1H, t, J = 7.5 Hz, H-4), 4.60 (1H, br d, J = 12.0 Hz, H-6′), 4.37 (1H, br t, J = 10.0 Hz, H-5′), 4.20 (1H, dd, J = 8.0, 12.0 Hz, H-6′), 4.17 (1H, dt, J = 7.5, 5.3 Hz, H-5), 3.98 (1H, dd, J = 5.3, 13.2 Hz, H-6), 3.93 (1H, dd, J = 5.3, 13.2 Hz, H-6), 3.84 (12H, s, 3″,5″-OMe and 3‴,6‴-OMe), 3.78 (6H, s″ H-3⁗,6⁗-OMe), 3.70 (1H, t, J = 10.0 Hz, H-3′), 3.63 (2H, s, H-1), 3.50 (1H, dd, J = 3.9, 10.0 Hz, H-2′), 3.30 (3H, t, J = 10.0 Hz, H-4′). ^{13}C NMR (methanol-d_4, 100 MHz): δ 169.2 (s, C-9‴), 167.8 (s, C-9⁗), 167.8 (s, C-9⁗), 151.1 (s, C-3⁗,5⁗), 149.4 (s, C-3‴,5‴), 149.3 (s, C-3″,5″), 148.4 (d, C-7″), 148.2 (d, C-7⁗), 147.1 (d, C-7‴), 139.9 (s, C-4⁗), 139.8 (s, C-4″), 139.6 (s, C-4‴), 126.6 (s, C-1″), 126.4 (s, C-1‴), 126.3 (s, C-1⁗), 116.0 (d, C-8‴), 115.2 (d, C-8″), 114.6 (d, C-8⁗), 107.1 (2C, d, C-2″,6″), 107.0 (2C, d, C-2‴,6‴), 106.9 (2C, d, C-2⁗,6⁗), 105.3 (s, C-2), 92.9 (d, C-1′), 82.8 (d, C-5), 76.9 (d, C-3), 76.4 (d, C-4), 75.0 (d, C-3′), 73.1 (d, C-2′), 72.9 (d, C-5′), 72.1 (d, C-4′), 66.0 (t, C-6′), 65.2 (t, C-1), 64.0 (t, C-6), 56.8 (18H, q, 3″,6″-OMe, 3‴,6‴-OMe and 3⁗,6⁗-OMe). FABMS m/z 983 $[M + Na]^+$.

1-*O*-(6″,9″,12″15″-Octadecateraenoyl)-3-*O*-β-D-galactopyranosyl Glycerol (7). Yellow oil; $[\alpha]_D$ −2.7° (*c* 1.0, MeOH). IR (KBr): ν_{max} 3383, 1743 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 5.28−5.40 (8H, m, H-6″, 7″, 9″, 10″, 12″, 13″, 15″, 16″), 4.22 (1H, d, J = 7.6 Hz, H-1′), 4.15 (1H, dd, J = 5.2, 11.6 Hz, H-3), 4.13 (1H, dd, J = 6.4, 11.6 Hz, H-3), 3.98 (1H, m, H-2), 3.90 (1H, dd, J = 5.2, 10.2 Hz, H-1), 3.81 (1H, d, J = 3.6 Hz, H-4′), 3.75 (2H, t, J = 5.6 Hz, H-6′), 3.65 (1H, dd, J = 4.4, 10.2 Hz, H-1), 3.53 (1H, dd, J = 7.6, 9.6 Hz, H-2′), 3.50 (1H, m, H-5′), 3.45 (1H, dd, J = 3.6, 9.6 Hz, H-3′), 2.81 (6H, t, J = 5.8 Hz, H-8″,11″,14″), 2.34 (2H, t, J = 7.7 Hz, H-2″), 2.06 (4H, m, H-5″, 17″), 1.63 (2H, m, H-3″), 1.37 (2H, m, H-4″), 0.98 (3H, t, J = 7.7 Hz, H-18). ^{13}C NMR (methanol-d_4, 100 MHz): δ 175.4 (s, C-1″), 128.2, 128.4, 128.8, 129.2, 130.0, 131.1, 131.6, 132.7 (each d, C-6″, C-7″, C-9″, C-10″, C-12″, C-13″, C-15″ and C-16″), 105.3 (d, C-1′), 76.7 (d, C-5′), 74.8 (d, C-3′), 72.5 (d, C-2′), 71.8 (t, C-1), 70.2 (d, C-4′), 69.6 (d, C-2), 66.6 (t, C-3), 62.4 (t, C-6′), 34.9 (t, C-2″), 29.8 (t, C-4″), 28.1 (2C, t, C-5″, 17″), 26.0, 26.5 (each t, C-8″, C-11″ and C-14″), 21.5 (t, C-3″), 14.6 (q, C-18″). FABMS m/z 513 $[M + H]^+$.

Fractionation of 1-Butanol Solubles. 1-Butanol solubles (13 g) were fractionated twice by MPLC equipped with an ODS column using a mixture of methanol−water followed by recycled HPLC with C-8 column using methanol−water twice to give two fractions, RS-B-1 and RS-B-2. RS-B-1 was further fractionated using recycled HPLC with C-8 column, and 1-feruloyl-β-D-glucopyranoside (**8**, 10.5 mg) (*16*) and 1-sinapoyl-β-D-glucopyranoside (**9**, 306 mg) (*17*) were obtained. From RS-B-2, β-D-(3-sinapoyl)frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (**10**, 262 mg) (*13*,*18*) was isolated.

1-Feruloyl-β-D-glucopyranoside (8). Yellow amorphous solid; $[\alpha]_D$ −42.1° (*c* 0.8, MeOH). UV (MeOH): λ_{max} 287 (3.7), 239 (3.4), 204 (3.7). IR (KBr): ν_{max} 3432, 1630 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.61 (1H, d, J = 15.9 Hz, H-7′), 7.20 (1H, d, J = 1.9 Hz, H-2′), 7.09 (1H, dd, J = 1.9, 8.3 Hz, H-6′), 6.81 (1H, d, J = 8.3 Hz, H-5′), 6.39 (1H, d, J = 15.9 Hz, H-8′), 5.56 (1H, d, J = 7.8 Hz, H-1), 3.89 (3H, s, 3′-OMe), 3.86 (1H, dd, J = 1.9, 12.2 Hz, H-6), 3.68 (1H, dd, J = 4.7, 12.2 Hz, H-6), 3.4−3.5 (overlapped with solvent peak, H-2 − H-5). ^{13}C NMR (methanol-d_4, 100 MHz): δ 167.7 (s, C-9′), 151.1 (s, C-4′), 148.2 (s, C-3′), 148.2 (d, C-7′), 127.5 (s, C-1′), 124.4 (d, C-6′), 116.6 (d, C-2′), 114.7 (d, C-8′), 111.9 (d, C-5′), 95.8 (d, C-1), 78.8 (d, C-5), 78.0 (d, C-3), 74.0 (d, C-2), 71.1 (d, C-4), 62.4 (t, C-6), 56.4 (q, 3′-OMe). FABMS m/z 357 $[M + H]^+$.

1-Sinapoyl-β-D-glucopyranoside (9). Yellow amorphous solid; $[\alpha]_D$ −61.0° (*c* 1.8, MeOH). UV (MeOH): λ_{max} 331 (3.0), 239 (3.9), 227 (3.9). IR (KBr): ν_{max} 3300, 1708 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.71 (1H, d, J = 16.1 Hz, H-7′), 6.92 (2H, s, H-2′,6′), 6.42 (1H, d, J = 16.1 Hz, H-8′), 5.58 (1H, d, J = 7.8 Hz, H-1), 3.87 (3H, s, 3″-OMe), 3.84 (1H, br d, J = 12.2 Hz, H-6), 3.69 (1H, dd, J = 7.7, 12.1 Hz, H-6), 3.4−3.5 (overlapped with solvent peak, H-2 − H-5). ^{13}C NMR (methanol-d_4, 100 MHz): δ 167.7 (s, C-9′), 149.5 (2C, s, C-3′,5′), 148.2 (d, C-7′), 139.9 (s, C-4′), 126.4 (s, C-1′), 114.7 (d, C-8′), 107.1 (2C, d, C-2′,6′), 95.8 (d, C-1), 78.8 (d, C-5), 78.0 (d, C-3), 74.1 (d, C-2), 71.1 (d, C-4), 62.1 (t, C-6), 56.9 (q, 3′-OMe). FABMS m/z 287 $[M + H]^+$.

β-D-(3-Sinapoyl)frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (10). Yellow amorphous solid; $[\alpha]_D$ −94.0° (*c* 3.6, MeOH). UV (MeOH): λ_{max} 309 (4.3), 239 (4.3), 224 (4.3). IR (KBr): ν_{max} 3368, 1698, 1634 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.66 (1H, d, J = 16.8 Hz, H-7″), 7.58 (1H, d, J = 16.8 Hz, H-7‴), 6.92 (2H, s, H-2‴,6‴), 6.87 (2H, s, H-2″,6″), 6.45 (1H, d, J = 16.8 Hz, H-8″), 6.44 (1H, d, J = 16.8 Hz, H-8‴), 5.50 (1H, d, J = 8.3 Hz, H-3), 5.50 (1H, d, J = 3.7 Hz, H-1′), 4.67 (1H, br d, J = 10.7 Hz, H-6′), 4.48 (1H, t, J = 8.3 Hz, H-4), 4.28 (1H, dt, J = 3.3, 8.0 Hz, H-5′), 4.21 (1H, dd, J = 8.0, 10.7 Hz, H-6′), 3.94 (1H, m, H-5), 3.90 (1H, m, H-6), 3.85 (12H, s, 3″,5″-OMe and 3‴,6‴-OMe), 3.83 (1H, m, H-6), 3.66 (1H, t, J = 9.4 Hz, H-3′), 3.61 (1H, d, J = 12.0 Hz, H-1), 3.59 (1H, d, J = 12.0 Hz, H-1), 3.47 (1H, dd, J = 3.7, 9.4 Hz, H-2′), 3.30

(1H, t, $J = 9.4$ Hz, H-4′). ^{13}C NMR (methanol-d_4, 100 MHz): δ 169.1 (s, C-9‴), 168.2 (s, C-9″), 149.4 (2C, s, C-3″,5″), 149.4 (2C, s, C-3‴,5‴), 147.9 (d, C-7″), 147.2 (d, C-7‴), 139.5 (s, C-4″), 139.5 (s, C-4‴), 126.6 (s, C-1‴), 126.5 (s, C-1″), 115.8 (d, C-8″), 115.5 (d, C-8‴), 107.0 (2C, d, C-2‴,6‴), 106.9 (2C, d, C-2″,6″), 104.8 (s, C-2), 92.6 (d, C-1′), 84.3 (d, C-5), 79.2 (d, C-3), 75.1 (d, C-3′), 74.1 (d, C-4), 73.1 (d, C-2′), 72.5 (d, C-5′), 71.9 (d, C-4′), 65.7 (t, C-1), 65.7 (t, C-6′), 63.8 (t, C-6), 56.8 (2C, q, C-3″,5″-OMe), 56.8 (2C, q, C-3‴,6‴-OMe). FABMS *m*/*z* 704 $[M]^+$.

Fractionation of Water Solubles. Water solubles (24 g) were fractionated by repeated MPLC with an ODS column. The resultant fraction was purified by HPLC to afford kaempferol-3,7-*O*-α-L-dirhamnopyranoside (**11**, 9 mg) (*19*) and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1−4)-β-D-glucopyranoside (**12**, 9 mg) (*20*).

Kaempferol-3,7-*O*-α-L-dirhamnopyranoside (11). Yellow amorphous solid; $[\alpha]_D$ −140.3° (*c* 1.0, MeOH). UV (MeOH): λ_{max} 337 (3.9), 265 (3.4), 228 sh (4.3), 203 (4.3). IR (KBr): ν_{max} 3417, 1650, 1634 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 7.78 (2H, d, $J = 9.1$ Hz, H-2′,6′), 6.93 (2H, d, $J = 9.1$ Hz, H-3′,5′), 6.73 (1H, d, $J = 2.0$ Hz, H-8), 6.47 (1H, d, $J = 2.0$ Hz, H-6), 5.55 (1H, d, $J = 1.2$ Hz, H-1″), 5.39 (1H, d, $J = 1.7$ Hz, H-1‴), 4.21 (1H, dd, $J = 1.2$, 3.2 Hz, H-2″), 4.00 (1H, dd, $J = 1.7$, 3.5 Hz, H-2‴), 3.81 (1H, dd, $J = 3.5$, 9.6 Hz, H-3‴), 3.71 (1H, dd, $J = 3.2$, 8.8 Hz, H-3″), 3.57 (1H, m, H-5‴), 3.46 (1H, t, $J = 9.6$ Hz, H-4‴), 3.32 (1H, t, $J = 8.8$ Hz, H-4″), 3.23 (1H, m, H-5″), 1.25 (3H, d, $J = 6.1$ Hz, H-6‴), 0.92 (3H, d, $J = 5.9$ Hz, H-6″). ^{13}C NMR (methanol-d_4, 100 MHz): δ 179.8 (s, C-4), 163.5 (s, C-7), 163.0 (s, C-5), 161.9 (s, C-4′), 159.9 (s, C-2), 158.1 (s, C-9), 136.4 (s, C-3), 132.0 (d, C-2′,6′), 122.3 (s, C-1′), 116.6 (2C, d, C-3′,5′), 108.0 (s, C-10), 103.5 (d, C-1‴), 100.5 (d, C-6), 99.9 (d, C-1″), 95.6 (d, C-8), 73.6 (d, C-4″), 73.1 (d, C-4‴), 72.1 (d, C-3″), 72.1 (d, C-3‴), 71.9 (d, C-2‴), 71.7 (d, C-2″), 71.3 (d, C-5″), 71.3 (d, C-5‴), 18.0 (q, C-6″), 17.7 (q, C-6‴). FABMS *m*/*z* 579 $[M + H]^+$.

Kaempferol-3-*O*-α-L-rhamnopyranosyl-(1−4)-β-D-glucopyranoside (12). Yellow amorphous solid; $[\alpha]_D$ −43.9° (*c* 1.8, MeOH). UV (MeOH): λ_{max} 346 (3.9), 266 (4.1), 223 sh (4.1), 204 (4.3). IR (KBr): ν_{max} 3414, 1645 cm^{-1}. ^{1}H NMR (methanol-d_4, 400 MHz): δ 8.07 (2H, d, $J = 9.1$ Hz, H-2′,6′), 6.89 (2H, d, $J = 9.1$ Hz, H-3′,5′), 6.76 (1H, d, $J = 2.3$ Hz, H-8), 6.46 (1H, d, $J = 2.3$ Hz, H-6), 5.56 (1H, d, $J = 1.7$ Hz, H-1″), 5.32 (1H, d, $J = 7.3$ Hz, H-1‴), 4.00 (1H, dd, $J = 1.7$, 3.3 Hz, H-2″), 3.82 (1H, dd, $J = 3.3$, 9.5 Hz, H-3″), 3.69 (1H, dd, $J = 1.6$, 11.1 Hz, H-6‴), 3.57 (1H, m, H-5″), 3.51 (1H, dd, $J = 6.2$, 11.1 Hz, H-6‴), 3.46 (1H, d, $J = 9.5$ Hz, H-4″), 3.42 (1H, t, $J = 7.3$ Hz, H-2‴), 3.40 (1H, t, $J = 7.3$ Hz, H-3‴), 3.31 (1H, t, $J = 7.3$ Hz, H-4‴), 3.19 (1H, m, H-5‴), 1.25 (3H, d, $J = 6.2$ Hz, H-6″). ^{13}C NMR (methanol-d_4, 100 MHz): δ 179.7 (s, C-4), 165.5 (s, C-7), 163.5 (s, C-5), 161.9 (s, C-4′), 159.6 (s, C-9), 158.0 (s, C-2), 135.5 (s, C-3), 132.3 (2C, d, C-2′,6′), 122.5 (s, C-1′), 116.2 (2C, d, C-3′,5′), 103.6 (s, C-10), 103.6 (d, C-1‴), 100.5 (d, C-6), 99.8 (d, C-1‴), 95.5 (d, C-8), 78.4 (d, C-5‴), 78.0 (d, C-3‴), 75.7 (d, C-2‴), 73.5 (d, C-4″), 72.0 (d, C-3″), 71.6 (d, C-2″), 71.4 (d, C-5″), 71.2 (d, C-4‴), 62.6 (t, C-6‴), 18.0 (q, C-6″). FABMS *m*/*z* 595 $[M + H]^+$.

RESULTS

Antioxidant Activity of the Methanol Extracts of Vegetables. Prior to the study of the antioxidant compounds contained in each vegetable, antioxidant activities of the methanol extracts of 11 kinds of vegetables were investigated using the BLM−Fe(III) method (*7*) at a concentration of 3 mg/mL. **Figure 2** shows the activities of the methanol extracts of vegetables. Three groups of activity were found in comparison with that of L-ascorbic acid groups with significantly strong activity (>1.3 VC), with almost equivalent activity to L-ascorbic acid, and with weak activity (<0.4 VC). As the results, *R. sativus* showed the strongest activity (1.8 VC) among the vegetables tested, followed by *B. rapa* var. *pervidis* (1.6 VC) and *Chrysanthemum coronarium* (1.5 VC).

Extraction and Fractionation of *R. sativus*. Because the methanol extract of *R. sativus* showed very strong antioxidant activity, we investigated its constituents as follows. The methanol extract was partitioned with hexane, ethyl acetate, and 1-butanol to give the corresponding solubles in the yields of 1.7, 9.2, 31, and 57%, respectively.

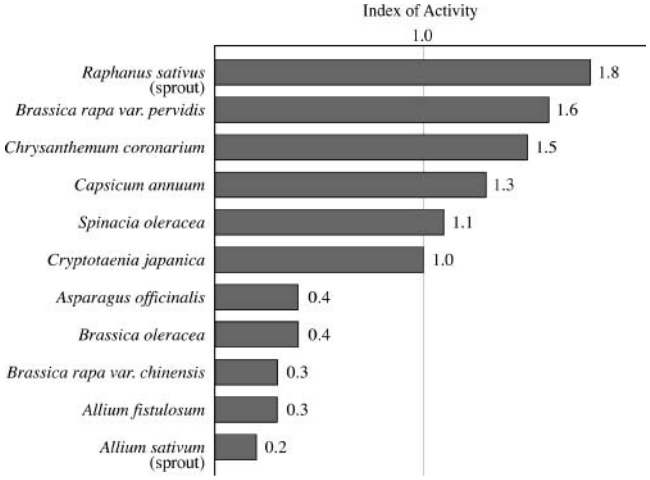

Figure 2. Antioxidant activity of vegetables. Activity was expressed as an index of activity as compared with L-ascorbic acid (1.0).

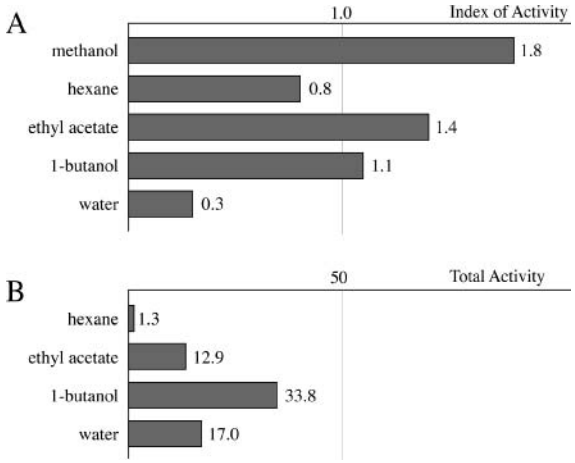

Figure 3. Antioxidant activity of solubles obtained from the methanol extract of *R. sativus*. (**A**) Index of activity of each soluble. (**B**) Total activity of each soluble as expressed the activity of methanol extract to be 100%.

Antioxidant Activity of the Solubles. Antioxidant activities of the solubles from *R. sativus* are exhibited in **Figure 3**. The ethyl acetate solubles and 1-butanol solubles showed comparatively high potency albeit not as strong as that of the methanol extract (**Figure 3A**). Furthermore, the total activity of each soluble was considerably lower than that of the methanol extract (**Figure 3B**).

Compounds Contained in Methanol Extract. Four kinds of solubles were respectively fractionated by repeated chromatography and gave 12 major compounds (**Figure 4**). The structures of the compounds were identified with the spectral data reported. From the hexane solubles, methyl linolenate (**1**) (*9*), linolenic acid (**2**) (*9*), phytol (**3**) (*10*), and methyl sinapate (**4**) (*11*) were isolated. Compound **4** was also isolated from the ethyl acetate solubles along with 1,2-disinapoyl-β-D-glucopyranoside (**5**) (*12*), β-D-(3,4-dipinapoyl)frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (**6**) (*13*), and 1-*O*-(6″,9″,12″,15″-octadecateraenoyl)-3-*O*-β-D-galactopyranosyl glycerol (**7**) (*14*, *15*). Three compounds, 1-feruloyl-β-D-glucopyranoside (**8**) (*16*), 1-sinapoyl-β-D-glucopyranoside (**9**) (*17*), and β-D-(3-sinapoyl)-frucofuranosyl-α-D-(6-sinapoyl)glucopyranoside (**10**) (*13*,*18*), were isolated from the 1-butanol solubles. Methyl sinapate (**4**) was reported to be a radical scavenger in *Brassica nigra* (brown

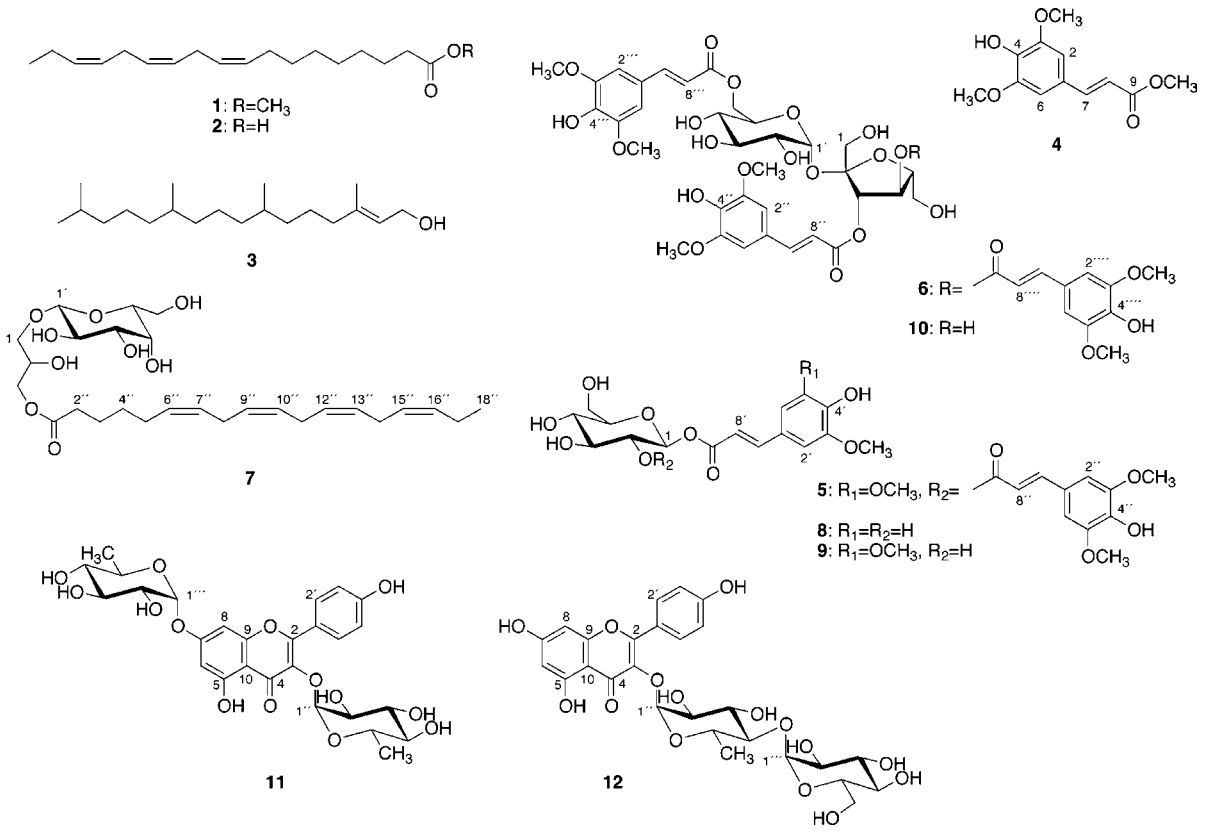

Figure 4. Structures of the compounds isolated from *R. sativus*.

Table 1. Antioxidant Activity of Compounds from *R. sativus*

entry	yield (mg)	activity (I_A)	entry	yield (mg)	activity (I_A)
1	274	0.4	**7**	102	1.7
2	131	0.6	**8**	10.5	2.3
3	11.6	1.6	**9**	306	2.3
4	35	2.6	**10**	262	2.7
5	100	2.4	**11**	9.0	2.2
6	132	2.6	**12**	9.0	2.0

mustard) (*11*). It is known that the amounts of sinapic acid esters, especially sinapoylated carbohydrates, in radish sprout vary according to the time and conditions of growth (*12*, *21*) and that those compounds distribute widely in Brassicaceaeous plants (*22*, *23*). However, compound **6** has been isolated only from *Securidaca longipedunculata* (Polygalaceae) (*13*), so this is the first isolation from Brassicaceae. In line with the results obtained here, sinapic acid esters were shown to be the major constituents in the methanol extract of radish sprout. Kaempferol-3,7-*O*-α-L-dirhamnopyranoside (**11**) (*19*) and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1−4)-*β*-D-glucopyranoside (**12**) (*20*) were isolated from the water solubles. These compounds were isolated from the Brassicaceae plant for the first time.

Antioxidant Activity of the Compounds. Antioxidant activities of the 12 compounds isolated from *R. sativus* were evaluated. As shown in **Table 1**, all compounds exhibited activity in comparison with L-ascorbic acid especially sinapic acid esters, which showed a high activity with a higher yield.

DISCUSSION

We measured the hydroxyl radical scavenging activity (antioxidant activity) of the methanol extract of 11 kinds of generally commercially available vegetables in Japan. As a result, we classified the vegetables into three categories. On the basis of these results, we speculate several patterns of compounds, which contribute to the high activity of the whole vegetables or their extract, as follows: (i) containing many kinds of comparatively highly active compounds in total high amounts, (ii) containing mainly a large amount of comparatively highly active compounds, and (iii) containing several kinds of significantly highly active compounds. In the course of our study of antioxidant active compounds in vegetables, we herein disclose the pattern to which each vegetable belongs.

Among the vegetables that we investigated, the constituents of radish sprout, which showed the highest activity, were examined. Unfortunately, solubles obtained from the methanol extract by partition with solvents did not reproduce the activity of the methanol extract. The reason for this has not been ascertained, but it is supposed to involve synergistic effects of the constituents. Then, we isolated 12 major compounds with antioxidant activity from the methanol extract of radish sprout (**Figure 4**). Among these compounds, sinapic acid esters were revealed to be the representative constituents. As Strack et al. reported (*12*), the amounts of these esters vary according to the stage of development of the radish sprout. In any event, these esters are estimated to be the major compounds in radish sprout and to contribute largely to the antioxidant activity. The antioxidant activity of most of the compounds, which are contained in large amounts in this plant, is higher as compared to that of L-ascorbic acid. Although the sum of the total activities (AA_T) of the constituents did not come up to the activity of the methanol extract, small amounts of various sinapic acid esters along with several kinds of flavonoids must be involved in the activity. On the basis of the antioxidant activity of the compounds, they are speculated to act synergistically and some minor constituents are supposed to enhance the activity of the major compounds in the methanol extracts. Accordingly, radish sprout may belong to the first pattern of our hypothesis

mentioned above. We are now investigating the constituents of garland chrysanthemum (*Chrysanthemum coronarium*) and spinach (*Spinacia oleracea*), which showed high and moderate antioxidant activity, respectively. From the preliminary results, garland chrysanthemum seems to possess highly active compounds and spinach contains moderately active compounds. We will report those compounds in the subsequent papers.

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