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Identification of Methanol-Soluble Compounds in Sesame and Evaluation of Antioxidant Potential of Its Lignans

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ABSTRACT: The methanol extract of sesame (*Sesamum indicum*) seeds was fractionated and purified with the assistance of conventional column chromatography to afford 29 compounds including seven furofuran lignans. Among these isolates, (+)-samin (**1**) was obtained from the natural source for the first time. In addition, (−)-asarinin (**30**) and sesamol (**31**) were generated by oxidative derivation from (+)-sesamolin (**2**) and (+)-sesamin (**3**), two abundant lignans found in sesame seeds. To evaluate their *in vitro* antioxidant potential, the seven isolated lignans (**1**−**7**) and the two derivatives (**30** and **31**) were examined for the scavenging activities on DPPH free radicals and superoxide anions. Moreover, the capability of chelating ferrous ions and reducing power of these sesame lignans were also measured. The results suggest that, besides the well-known sesamolin and sesamin, the minor sesame lignans (+)-(7*S*,8′*R*,8*R*)-acuminatolide (**5**), (−)-piperitol (**6**), and (+)-pinoresinol (**7**) are also adequate active ingredients and may be potential sources for nutritional and pharmacological utilization.

KEYWORDS: antioxidant, lignans, free radical scavenging, sesame, superoxide anion inhibition

INTRODUCTION

Sesame seeds and sesame oil have long been used as health foods in Asian countries. In comparison with most other edible oils extracted from diverse seeds, sesame oil is extremely stable due to the effective antioxidant activities presumably attributed to its abundance of lipid-soluble furofuran lignans.[1] Sesamin and sesamolin, the two major furofuran lignans, were found to suppress lipid peroxidation in erythrocytes, to inhibit intestinal absorption of cholesterol and hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity, to prevent chemically induced mammary cancer, to inhibit Δ^5-desaturase and chain elongation of C18 fatty acids, to protect hypoxic neuronal and PC12 cells by suppressing ROS generation and MAPK activation, to exhibit antihypertensive effects, and to enhance liver detoxification of carbon tetrachloride and ethanol.[2−10] The pharmacological effects of sesamin and sesamolin seem to be directly or indirectly correlated to their antioxidant and free radical scavenging activities that may suppress oxidative stresses *in vivo*.[11]

Besides abundant sesamin and sesamolin, several minor furofuran lignans were also found in sesame seeds.[12] Possessing the same furofuran skeleton as sesamin and sesamolin, these minor sesame lignans displayed antioxidant potentials as examined by the free radical scavenging, reducing power, and lipid peroxidation assays.[13−16] In contrast with furofuran lignans, other compounds in sesame seeds do not attract much attention, and thus they have not been well-characterized so far.

In the present study, we first purified and characterized 29 compounds in the methanol extract of sesame seeds. Among these isolates, five minor furofuran lignans along with the abundant sesamin and sesamolin were identified. Moreover, oxidation of sesamin and sesamolin led to the generation of their derivatives. *In vitro* antioxidant activities of the purified lignans and their derivatives were evaluated.

MATERIALS AND METHODS

General. All the chemicals were purchased from Merck KGaA (Darmstadt, Germany) unless specifically indicated. Melting points of purified compounds were determined by a FISHER Scientific melting point measuring apparatus without corrections. The UV spectra were obtained on a GBC Cintra 101 UV−vis spectrophotometer. The IR spectra were obtained, as KBr disks, on a VARIAN Scimitar FTS-2000 FT-IR spectrometer. Optical rotations were measured with the ATAGO AP-300 automatic polarimeter. The mass and high-resolution mass spectra were obtained on a Bruker APEX II mass spectrometer. ^{1}H and ^{13}C NMR, COSY, NOESY, HMQC, and HMBC spectra were recorded on a Bruker AV-500 NMR spectrometer with tetramethylsilane as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments, and all chemical shifts were reported in parts per million (ppm, δ). Column chromatography and thin layer chromatography (TLC) were performed on silica gels (Kieselgel 60, 70−230 mesh and 230−400 mesh, Merck KGaA) and precoated Kieselgel 60 F 254 plates (Merck KGaA), respectively.

Plant Materials. The seeds of sesame (*Sesamum indicum* L. Tainan-1) were provided and authenticated by Dr. Yiu of the Corn Research Center, Tainan District Agricultural Research and Extension Station. A voucher specimen (PCKuo_2006001) was deposited in the herbarium of Department of Biotechnology, National Formosa University, Yunlin, Taiwan.

Extraction and Purification. Sesame seeds (1 kg) were powdered and exhaustively extracted with methanol under reflux (10 L × 5 × 8 h), and the combined extracts were concentrated under reduced pressure to give a dark brown syrup (200 g). The crude extract was successively

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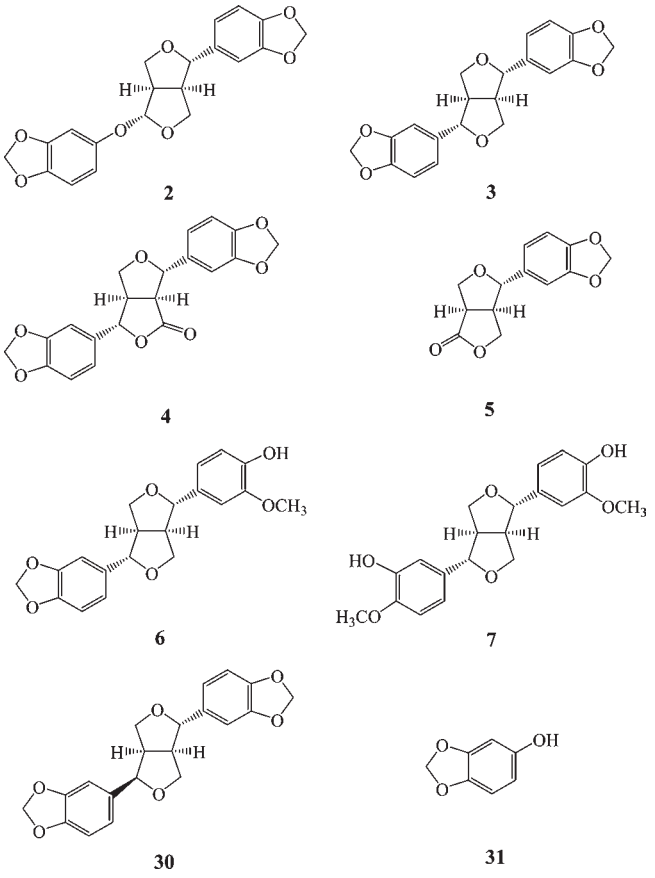

Figure 1. The chemical structures of compounds **2**−**7**, **30**, and **31**.

partitioned with *n*-hexane and ethyl acetate to afford *n*-hexane (50 g), ethyl acetate (10 g), and water extracts (140 g).

The *n*-hexane extract was applied to a silica gel column, and then eluted with *n*-hexane and a step gradient of ethyl acetate (100:1 to 1:1) to afford 8 fractions. Fraction 2 was subjected to recrystallization with chloroform and methanol to yield a mixture of *β*-sitosterol and stigmasterol (3.9 g).[17,18] Fraction 3 was further resolved on a silica gel column eluted with *n*-hexane and ethyl acetate (20:1) to give a mixture of *β*-sitostenone and stigmasta-4,22-dien-3-one (5 mg, R_f = 0.30)[17,19] and *β*-amyrenone (2 mg, R_f = 0.25).[20] Fraction 5 was applied to a silica gel column, and then eluted with the mixture of *n*-hexane and acetone (20:1) to afford (+)-samin (**1**) (500 mg, R_f = 0.35)[21] and (+)-sesamolin (**2**) (5.9 g, R_f = 0.30)[22] (Figure 1). Fraction 6 was separated by silica gel column chromatography with the solvent mixture of *n*-hexane and ethyl acetate (50:1) and a step gradient with ethyl acetate to yield a mixture of 6*β*-hydroxystigmast-4-en-3-one and stigmasta-4,22-dien-6*β*-ol-3-one (3 mg, R_f = 0.40),[23,24] (+)-sesamin (**3**) (15.7 g, R_f = 0.35),[17] and (−)-aptosimon (**4**) (8 mg, R_f = 0.20),[25] respectively. Fraction 7 was subjected to recrystallization with chloroform and methanol to afford *β*-sitosterol-3-*O*-*β*-glucopyranoside (720 mg).[17]

The ethyl acetate extract was applied to a silica gel column, and then eluted with *n*-hexane and a step gradient of acetone (50:1 to 1:1) to afford 5 fractions. Fraction 2 was further separated by repeated column chromatography over silica gel eluted with *n*-hexane and ethyl acetate gradients followed by purification with preparative TLC on silica gel to yield methylparabene (3 mg, R_f = 0.50),[26] methyl vanillate (10 mg, R_f = 0.40),[26] and aurantiamide acetate (5 mg, R_f = 0.30).[27] Fraction 3 was subjected to silica gel column chromatography eluted with a mixture of *n*-hexane and acetone (30:1) to result in four subfractions. Subfraction 3-1 was purified by preparative TLC eluted with *n*-hexane and acetone (50:1) to yield vanillin (3 mg, R_f = 0.35)[26] and methyl ferulate (5 mg, R_f = 0.25).[28] Subfraction 3-2 was recrystallized with acetone to afford methyl 3-indolecarboxylate (8 mg).[29] Subfraction 3-3 was resolved in a silica gel column eluted with *n*-hexane and ethyl acetate gradients followed by purification with preparative TLC on silica gel to yield vanillic acid (25 mg, R_f = 0.40)[26] and (+)-(7*S*,8′*R*,8*R*)-acuminatolide (**5**) (17 mg, R_f = 0.30).[30] Subfraction 3-4 was recrystallized with acetone to result in methyl 3,4,5-trimethoxybenzoic acid (9 mg).[31] Fraction 4 was resolved on a silica gel column eluted with *n*-hexane and chloroform (20:1) followed by the preparative TLC to afford 4-hydroxy-3,5-dimethoxybenzaldehyde (9 mg, R_f = 0.45),[17] (−)-piperitol (**6**) (13 mg, R_f = 0.40),[32] and 1*H*-indole-3-carbaldehyde (37 mg, R_f = 0.25).[33] Fraction 5 was purified with the aid of silica gel column chromatography eluted with chloroform and acetone (50:1) to yield (+)-pinoresinol (**7**) (7 mg, R_f = 0.30).[34]

The water-soluble extract was applied to a reversed-phase Diaion HP-20 column eluted with water and methanol gradients to afford 6 fractions. Fractions 2 and 3 were subjected to C-18 column chromatography eluted with water and methanol gradients and further recrystallization with methanol to yield uridine (20 mg)[35] and adenosine (7 mg),[35] respectively. Fraction 4 was purified by silica gel column chromatography eluted with chloroform and acetone gradients followed by preparative TLC to afford nicotinic acid (8 mg, R_f = 0.20).[26] Fraction 5 was purified with the aid of silica gel column chromatography eluted with chloroform and acetone gradient solvent mixture, and then recrystallized with methanol to yield mudanoside A (2 mg, R_f = 0.30).[36]

Spectral Data of (+)-Samin (1). **1**: colorless needles; mp 183−185 °C; $[\alpha]_D^{25}$ +200 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 287 (2.69), 238 (2.83), 203 (4.21) nm; IR (KBr) ν_{max} 3400, 2908, 1728, 1594, 1489, 1242, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 6.86 (1H, d, *J* = 1.3 Hz, H-2), 6.81 (1H, br d, *J* = 8.0 Hz, H-6), 6.77 (1H, d, *J* = 8.0 Hz, H-5), 5.95 (2H, s, OCH_2O), 5.38 (1H, br s, H-7′), 4.37 (2H, m, H-7 and −9α), 4.17 (1H, dd, *J* = 9.1, 6.0 Hz, H-9′α), 3.91 (1H, dd, *J* = 9.1, 0.8 Hz, H-9′*β*), 3.57 (1H, dd, *J* = 8.2, 8.0 Hz, H-9*β*), 3.01 (1H, ddd, *J* = 8.7, 8.4, 8.1 Hz, H-8′), 2.84 (1H, m, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 148.0 (C-3), 147.3 (C-4), 134.6 (C-1), 119.6 (C-6), 108.1 (C-5), 106.5 (C-2), 102.9 (C-1′), 101.0 (OCH_2O), 87.0 (C-7), 71.3 (C-9), 69.4 (C-3′), 52.8 (C-8), 52.7 (C-2′). ESI-MS *m/z* (%): 273 ($[M+Na]^+$, 100); HR-ESI-MS *m/z* 273.0737 $[M+Na]^+$ (Calcd for $C_{13}H_{14}O_5Na$, 273.0739).

Oxidation of Sesamolin (2) and Sesamin (3). Sesamin (**3**) (1 mmol) was added to a solution of $K_2Cr_2O_7$ (1.1 mmol), concentrated H_2SO_4 (0.1 mL), and acetone (5 mL). The reaction mixture was stirred at ambient temperature over a period of 24 h until no more starting material was detected by TLC. After removal of the solvent under reduced pressure, the crude reaction mixture was afforded as pale yellow oils. The crude product was purified by silica gel column chromatography (230−400 mesh) using the mixing solvent pairs of *n*-hexane/EtOAc (70:30) to afford (−)-aptosimon (**4**) (0.7 mmol, 70%). Under the same reaction condition by replacing $K_2Cr_2O_7$ with $KMnO_4$, sesamin was oxidized to afford (−)-asarinin (**8**) (0.65 mmol, 65%). Similarly, sesamolin was oxidized by either $K_2Cr_2O_7$ or $KMnO_4$ to afford sesamol (**9**) (0.4 mmol, 40%).

Assay of Scavenging Activity against Diphenyl Picrylhydrazyl (DPPH) Radical. The DPPH free radical scavenging assay was executed according to the reported method.[37] A methanol solution of 1,1-diphenyl-2-picrylhydrazyl radical (200 μM) was incubated with an equal volume of each test sample for 30 min, and then recorded for the absorbance at 517 nm. The DPPH radical scavenging activities of the sample solutions were expressed as inhibition percentage and calculated by using the following equation: inhibition percentage (%) = [1 − (absorbance of samples at 517 nm)/(absorbance of control at 517 nm)] × 100%.

Superoxide Anion Inhibition Assay. The superoxide anion scavenging activities of sesame lignans were assayed by measuring the inhibition of nitro-blue tetrazolium (NBT) reduction by NADH in the presence of phenazine methosulfate (PMS).[38] The incubation mixture contained 73 μM NADH, 15 μM PMS, 50 μM NBT in 20 mM K-phosphate buffer, pH 7.4 and the test compounds of various

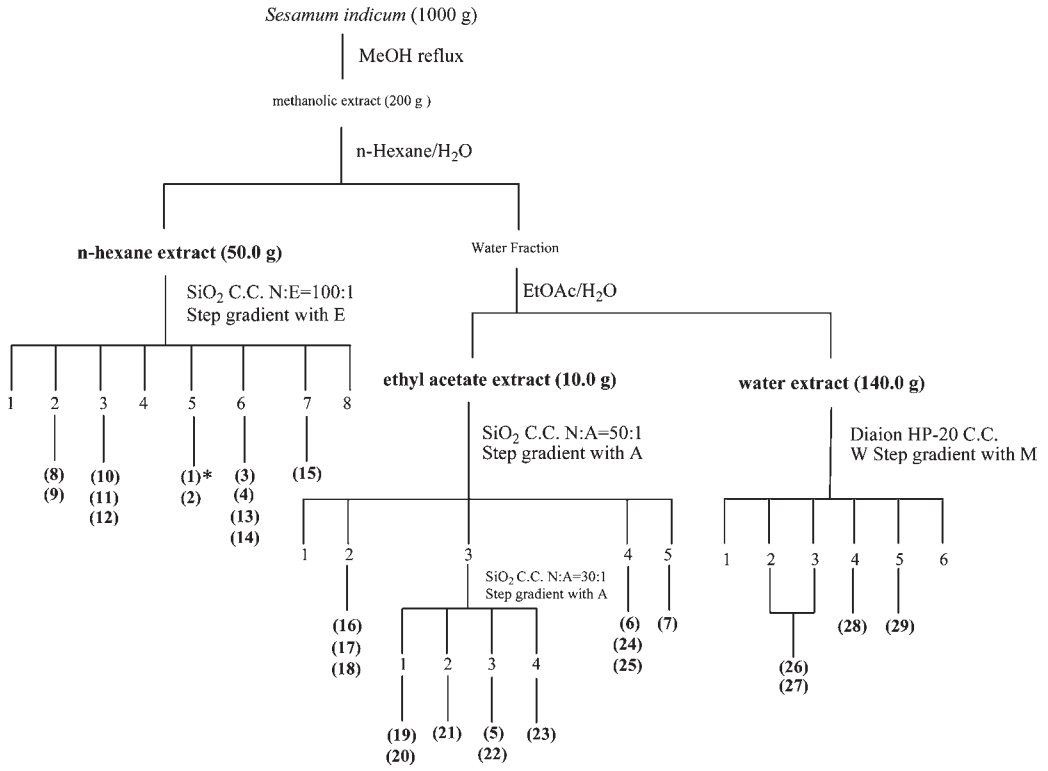

Figure 2. Scheme for purification of methanol-soluble compounds from sesame seeds.

concentrations. Absorbance variations were determined at 560 nm, measuring the initial rate of superoxide-induced NBT reduction.

Chelation of Ferrous Ions. The capability of chelating ferrous ions was determined for sesame lignans by the reported method with some modifications.[39] Tested samples prepared in 100 μL of 1 mM solution were added with 50 μL of 2 mM ferrous chloride. The reaction was initiated by addition of 100 μL of 5 mM ferrozine, and the mixture was shaken vigorously. After sitting at room temperature for 10 min, the absorbance of the reaction mixture was measured at 562 nm. The relative capability of chelating ferrous ions was derived from the formula [1 − (sample absorbance at 562 nm)/(control absorbance at 562 nm)] × 100 (%).

Reducing Power Assay. The reducing power of sesame lignans was examined according to a reported method with some modifications.[40] Tested samples prepared in 100 μL of 0.2 M phosphate buffer, pH 6.6 were added with 100 μL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, combined with 100 μL of 10% trichloroacetic acid, and then centrifuged at 650*g* for 10 min. The supernatant was mixed with 200 μL of distilled water and 40 μL of 0.1% ferric chloride. The absorbance of the resulting solution was determined at 700 nm; higher absorbance value indicated greater reducing power.

Statistical Analysis. Data were analyzed with Duncan's multiple range tests in a standard statistical package (SAS/STAT) (Version 9.1, SAS institute Inc., NC, USA). Statistical significance was assessed for each plot.

RESULTS AND DISCUSSION

Purification and Identification of Methanol-Soluble Compounds in Sesame Seeds. The methanol extract of powdered sesame seeds was suspended in water and partitioned with *n*-hexane and ethyl acetate, successively to afford *n*-hexane, ethyl acetate, and water-soluble fractions (Figure 2). Further purification of each fraction by a combination of conventional chromatographic techniques yielded twenty-nine compounds (Table 1). Among these isolates, the structure of compound **1** was determined to be (+)-samin by the NMR and mass spectral elucidations. Compounds **2**−**29** were known constituents as identified by comparison of their spectral and physical data with those reported in the literature. Compounds **2**−**7** were also classified as furofuran lignans according to their chemical structures.

Structural Determination of (+)-Samin (1). Compound **1** was purified as optically active colorless needles with mp 183−185 °C and $[\alpha]_D^{25}$ +200 (*c* 0.1, $CHCl_3$). The UV spectrum of compound **1** in methanol exhibited characteristic absorption maxima of a lignan skeleton at 287, 238, and 203 nm.[21] The IR absorption bands at 3400 and 1489 cm^{-1} displayed the presence of hydroxyl group and carbon−carbon double bond, respectively. In the ^1H NMR spectrum, a set of three mutually coupled ABX-type proton signals at δ 6.86 (1H, d, J = 1.3 Hz), 6.81 (1H, br d, J = 8.0 Hz), and 6.77 (1H, d, J = 8.0 Hz) was indicative of a trisubstituted benzene ring. One typical dioxygenated methylene group was located at δ 5.95 (2H, s). In addition, one broad singlet at δ 5.28 (1H) and one multiplet at δ 4.37 (1H) were characteristic of the H-7′ and H-7 of sesamolinol-type lignans.[12] Six proton signals resonating at δ 4.37 (1H, m), 3.98 (1H, dd, J = 9.0, 8.1 Hz), 3.91 (1H, dd, J = 9.0, 8.7 Hz), 3.57 (1H, dd, J = 8.2, 8.0 Hz), 3.01 (1H, ddd, J = 8.7, 8.4, 8.1 Hz, H-8′), and 2.84 (1H, m, H-8) accounted for two aliphatic methines and two oxygenated methylenes. These spectral data were the same as for the previously reported compound, (+)-samin.[21] The full assignments of ^1H and ^{13}C NMR signals were determined from the NOESY and HMBC spectra (Figure 3). From the above spectral data, the chemical structure of compound **1** was concluded to be (+)-samin. Although chemical synthesis of (+)-samin had already been reported previously,[21] this compound was isolated from the natural source for the first time.

Oxidative Derivation of Sesamolin (2) and Sesamin (3). Among the twenty-nine isolated compounds from sesame seeds, two furofuran lignans, sesamolin (**2**) and sesamin (**3**), were found

Table 1. Relative Contents of Compounds Found in Methanol Extract of Sesame Seeds

	compd	rel contents (mg/1 kg sesame)	refs
1	(+)-samin	500	21
2	(+)-sesamolin	5900	22
3	(+)-sesamin	15700	17
4	(−)-aptosimon	8	25
5	(+)-(7*S*,8′*R*,8*R*)-acuminatolide	17	30
6	(−)-piperitol	13	32
7	(+)-pinoresinol	7	34
8	*β*-sitosterol	3900 (**8** and **9**)	17
9	stigmasterol		18
10	*β*-sitostenone	5 (**10** and **11**)	17
11	stigmasta-4,22-dien-6*β*-ol-3-one		19
12	*β*-amyrenone	2	20
13	6*β*-hydroxystigmast-4-en-3-one	5 (**13** and **14**)	23
14	stigmasta-4,22-dien-6*β*-ol-3-one		24
15	*β*-sitosterol-3-*O*-*β*-glucopyranoside	720	17
16	methylparabene	3	26
17	methyl vanillate	10	26
18	aurantiamide acetate	5	27
19	vanillin	3	26
20	methyl ferulate	5	28
21	3-indolecarboxylate	8	29
22	vanillic acid	25	26
23	methyl 3,4,5-trimethoxybenzoic acid	9	31
24	4-hydroxy-3,5-dimethoxybenzaldehyde	9	17
25	1*H*-indole-3-carbaldehyde	37	33
26	uridine	20	35
27	adenosine	7	35
28	nicotinic acid	8	26
29	mudanoside A	2	36

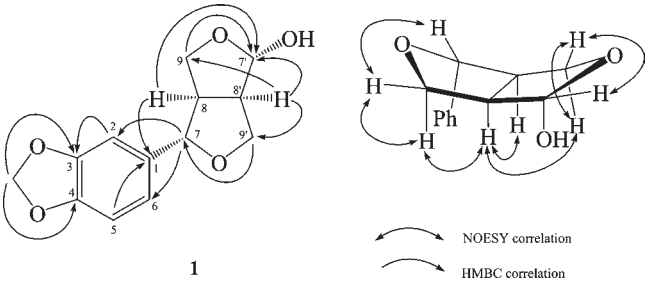

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Figure 3. The chemical structure and significant HMBC and NOESY correlations of **1**.

relatively abundant (Table 1). Moreover, these two furofuran lignans have been demonstrated to possess several biological activities, such as neuroprotective effect, antihypertensive effect, suppression of lipid peroxidation, and liver detoxification of carbon tetrachloride and ethanol.[2−10] To generate more derivatives, these two sesame lignans were subjected to further oxidation with two different oxidizing agents. The outcomes showed that sesamin (**3**) was oxidized by $K_2Cr_2O_7$ under acidic conditions to afford (−)-aptosimon (**4**), which resulted from the oxidation of the methylene group in the furofuran ring. However, (−)-asarinin (**30**) was yielded due to the epimerization of the furofuran ring when the oxidizing agent was replaced with $KMnO_4$. In contrast, sesamolin (**2**) was oxidized to break the C−O linkage of the furan ring to afford sesamol (**31**) without any other products under the same reaction conditions with either $K_2Cr_2O_7$ or $KMnO_4$ as the oxidizing agent.

Table 2. DPPH Free Radical Scavenging Activity of Compounds 1−7, 30, and 31

compd (500 *μ*M)	IC_{50} (*μ*M)	inhibn percentage (%)
1	−[a]	40.7 ± 3.0
2	−	29.0 ± 3.7
3	−	27.2 ± 3.1
4	−	39.5 ± 0.3
5	224.1 ± 0.6	77.4 ± 0.5
6	49.5 ± 1.2	84.9 ± 0.7
7	34.5 ± 1.1	88.6 ± 0.6
30	−	43.3 ± 2.2
31	37.3 ± 2.9	88.3 ± 1.4
vitamin C	38.6 ± 1.8	89.7 ± 0.1

[a] Not determined.

Free Radical Scavenging Activities of the Purified and Synthetic Lignans. Radical scavenging activities of compounds **1**−**7** as well as those of the synthetic derivatives **30** and **31** were evaluated in two assay systems by measuring their scavenging

Table 3. Superoxide Anion Scavenging Activity of Compounds 1−7, 30, and 31

	inhibn percentage (%)				
compd	32.25 μM	62.5 μM	125 μM	250 μM	500 μM
1	14.24 ± 0.58	21.66 ± 1.86	23.00 ± 4.57	36.57 ± 0.88	44.18 ± 1.64
2	11.26 ± 7.19	16.46 ± 2.89	21.37 ± 3.72	28.59 ± 6.07	22.23 ± 3.35
3	12.13 ± 2.68	14.34 ± 0.33	17.90 ± 0.73	26.18 ± 0.93	34.65 ± 0.60
4	11.07 ± 1.50	17.52 ± 2.67	19.15 ± 3.50	36.57 ± 2.52	40.81 ± 0.58
5	27.82 ± 1.89	28.78 ± 3.94	31.67 ± 3.19	41.39 ± 0.58	43.50 ± 0.44
6	16.07 ± 1.67	27.62 ± 0.60	28.78 ± 0.60	36.00 ± 1.30	43.98 ± 1.53
7	21.75 ± 1.76	22.04 ± 1.50	28.01 ± 8.58	37.73 ± 0.44	40.13 ± 0.83
30	20.40 ± 4.83	23.68 ± 2.17	33.49 ± 2.19	34.46 ± 0.76	39.75 ± 0.17
31	22.23 ± 5.25	25.89 ± 4.84	31.95 ± 0.17	35.03 ± 0.76	40.62 ± 1.20
BHT	22.23 ± 3.77	23.68 ± 4.13	22.14 ± 4.80	28.59 ± 6.09	29.26 ± 0.87

Table 4. Reducing Power of Compounds 1−7, 30, and 31

compd (113 μM)	inhibn percentage (%)
1	5.0 ± 1.7
2	20.6 ± 1.0
3	9.1 ± 2.2
4	4.7 ± 0.7
5	2.4 ± 1.5
6	6.3 ± 2.0
7	12.4 ± 3.2
30	9.6 ± 3.9
31	34.8 ± 1.6
vitamin C	52.6 ± 1.6

activities to DPPH free radicals and superoxide anions, respectively. In the former assay, compounds **5**, **6**, 7, and **31** exhibited significant DPPH free radical scavenging activity with inhibition percentage higher than 50% (Table 2). Among these four compounds, (+)-pinoresinol (7) and sesamol (**31**) displayed relatively strong antioxidant activities with their IC_{50} values (34.5 ± 1.1 and 37.3 ± 2.9 μM, respectively) slightly lower or equivalent to that of the reference compound vitamin C (38.6 ± 1.8 μM). In the latter assay, BHT (butylated hydroxytoluene), an antioxidant food additive was used a reference compound for the evaluation of inhibitory activity on superoxide anions. The results showed that sesamolin (**2**) displayed scavenging activity comparable to that of BHT while the other eight compounds exhibited better scavenging activity, particularly when the concentrations of the tested compounds increased in the range from 32.25 to 500 μM (Table 3). It is suggested that besides the abundant sesamolin (**2**) and sesamin (**3**), their oxidative derivatives and other minor sesame lignans may also be of nutritional and pharmacological importance.

Chelating Ferrous Ions and Reducing Power of Sesame Lignans. The capability of chelating ferrous ions of the purified lignans **1**−**7** and the synthetic derivatives **30** and **31** were measured. None of the examined compounds exhibited significant chelation of ferrous ions at the tested concentration of 400 μM (data not shown). Moreover, the reducing power of these sesame lignans was measured. Only sesamol (**31**) displayed weak reducing power at 113 μM in comparison with the positive control, vitamin C (Table 4). Taken together, the mechanisms possibly involved in the protections of sesame lignans against diseases are related to their scavenging activities on free radicals caused by oxidative stresses.

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Author Contributions

‡These two authors had the same contributions.

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