J Food Sci Nutr
Vol 10, p 326~332 (2005)

J Food Science and Nutrition

Radical Scavenging Activities of Phenolic Compounds Isolated from Mulberry (*Morus* spp.) Cake

Young-Woong Shin, Seong-Kwon Lee, Yun-Ju Kwon, Soon-Jae Rhee and Sang-Won Choi†

Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongbuk 712-702, Korea

Abstract

A methanol extract of mulberry cake prepared from mulberry fruits (*Morus* spp.) was shown to have strong scavenging activities against DPPH, superoxide and hydroxyl radicals. Eleven phenolic compounds were isolated from the mulberry cake by a combination of Diaion HP-20, silica gel (or polyamide), Sephadex LH-20 column chromatographies, preparative HPLC and TLC. Their chemical structures were characterized as procatechuic acid (PCA), caffeic acid (CA), cyanidin 3-*O*-β-D-glucopyranoside (CyG) and cyanidin 3-*O*-β-D-rutinoside (CyR), rutin (RT), isoquercitrin (IQT), astragalin (AG), quercetin (QT), morin (MR), dihydroquercetin (DHQ), and 4-prenylmoracin (PM) by spectral analysis and the published data. Most of the phenolic constituents were effective scavengers of DPPH, superoxide and hydroxyl radicals, and especially caffeic acid and 4-prenylmoracin showed potent superoxide and hydroxyl radical scavenging activity, in which their activities were higher than that of the well-known antioxidant, BHT ($p<0.05$). Dehydroquercetin and quercetin also exhibited strong superoxide and hydroxyl radical scavenging activities. These results suggest that mulberry cake containing antioxidant phenolic compounds may be useful as natural antioxidants in functional foods and cosmetics.

Key words: mulberry (*Morus* sp.) cake, radical scavenging activity, phenolic compounds

INTRODUCTION

Much attention has recently been focused on natural antioxidants capable of inhibiting reactive oxygen radical-mediated lipid peroxidation which is closely associated with several pathological disorders such as cancer, atherosclerosis, and aging (1,2). Natural polyphenolic compounds are of particularly great interest as nutraceutical ingredients for prevention of several degenerated diseases caused by reactive oxygen species, such as singlet oxygen, superoxide and hydroxyl radicals (3,4).

Mulberry (*Morus* spp.) fruit, "Sangsimja", has been used as folk medicine for treatment of diabetes, baldness, hangover, hypertension and inflammation, etc. (5,6). Recently, mulberry fruit has been recognized to possess a variety of biological effects such as antidiabetic (7,8), antioxidant (9-11), anti-inflammatory (10), and antihyperlipidemic (12) activities. Anthocyanins, flavonoids, polyhydroxy-alkaloids and γ-aminobenzoic acid (GABA) are major active compounds responsible for the physiological activities of mulberry fruits (8,13). However, systematic studies screening for phenolic antioxidants from mulberry fruit are still not carried out.

Levels of several phytochemical constituents in mulberry fruits could be affected by maturity, cultivar and processing (14-16). Particularly, because mulberry fruits possess large amounts of anthocyanin pigment which is heat labile, a non-thermal minimally processed mulberry juice using a membrane filteration or filter aids needs to be developed (17). Mulberry cake, a byproduct of minimally processed mulberry juice, possessed high amounts of phenolics with radical scavenging activity stronger than mulberry juice (18).

The objective of this study was to isolate and identify phenolic compounds from mulberry cake, and to evaluate their radical scavenging activity against DPPH, superoxide and hydroxy radicals using *in vitro* assays.

MATERIALS AND METHODS

Materials

Mulberry fruits of the Chongilppong (*M. alba* L.) tree were directly harvested in the middle of June, 2004 from a farm in Yeongcheon, Gyeongbuk, Korea. The mulberry fruits were freeze-dried and stored at -18°C until used.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), xanthine oxidase (EC 1.2.3.2), xanthine, nitrobluetetrazolium chlo-

†Corresponding author. E-mail: swchoi@cu.ac.kr
Phone: +82-53-850-3525, Fax: +82-53-850-3504

ride (NBT), thiobarbituric acid (TBA), H_2O_2, bovine serum albumin (BSA), trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO) and NMR solvents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Butylated hydroxytoluene (BHT) and $FeSO_4 \cdot 7H_2O$ were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Sodium phosphate dibasic 12 hydrate and potassium phosphate monobasic were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). All solvents and reagents used in this study were of the first analytical grade.

Isolation and identification of phenolic compounds

The freeze-dried mulberry fruit (100 g) was homogenized twice with 0.5% TFA in D-H_2O (200 mL) and filtered with cheesecloth. The residue was washed continuously with 0.5% TFA in D-H_2O to remove anthocyanin pigment, and squeezed to yield mulberry cake. The mulberry cake was extracted twice with MeOH (1 L) under reflux at 70~80°C for 2 hr, filtered and evaporated under reduced pressure. The MeOH extract (10.55 g) was solubilized in 0.1% TFA in 10% aq. MeOH (300 mL) and loaded onto a Diaion HP-20 (Mitsubishi Chem. Co., Tokyo, Japan) column (5.5×50 cm) pre-equilibrated with 10% aq. MeOH. The column was eluted successively with 20% (2 L), 40% (2 L), 60% (1.5 L), 80% (1 L) and 100% MeOH (1 L), and each fraction was then concentrated to yield 20% MeOH fr. (3.13 g), 40% MeOH fr. (1.76 g), 60% MeOH fr. (1.54 g), 80% MeOH fr. (1.21 g) and a 100% MeOH fr. (0.83 g). The 20% MeOH fr. (3.13 g) was chromatographed on silica gel (70~230 mesh, Merck, Damstadt, Germany) column (5.5×30 cm) with $CHCl_3$-MeOH-H_2O (50:50:10, v/v) as an eluent, to afford five fractions; Fr. 1 (0.89 g), Fr. 2 (0.21 g), Fr. 3 (0.11 g), Fr. 4 (0.29 g) and Fr. 5 (0.45 g). The Fr. 2 and Fr. 4 were further chromatographed on a Sephadex LH-20 (Parmacia Biotech., Uppsala, Sweden) column (2.5×80 cm) with 80% aq. MeOH, to give pure compound **1** (Comp. **1**, 48 mg) and compound **2** (Comp. **2**, 40 mg), respectively. The 40% MeOH fr. (1.76 g), a predominantly anthocyanin-containing fraction, was successively fractionated by Polyamide C-200 (75~150 μm, Wako Pure Chem. Ind. Ltd., Osaka, Japan) column (4.0×50 cm) chromatography and prep-HPLC (Waters Delta Prep-4000, Waters, USA) (16), and thereby isolating two anthocyanin pigments (Comp. **3**, 68 mg; Comp. **4**, 18 mg). The 60% MeOH fr. (1.54 g) was chromatographed on silica gel column with $CHCl_3$-MeOH-H_2O (65:35:10, v/v) as an eluent, to give five fractions; Fr. 1 (21 mg), Fr. 2 (78 mg), Fr. 3 (101 mg), Fr. 4 (152 mg) and Fr. 5 (20 mg). The Fr. 2-Fr. 4 were further chromatographed on a preprative silica gel TLC (Merck, Damstadt, Germany) with $CHCl_3$-MeOH-H_2O (65:35:10, v/v), and separated pure compound **5** (Comp. **5**, 14 mg), compound **6** (Comp. **6**, 28 mg), and compound **7** (Comp. **7**, 64 mg), respectively. The 80% MeOH fraction (1.21 g) was also subjected to silica gel column and prep-TLC with $CHCl_3$-MeOH-HOAc (30:10:0.1, v/v), and isolated as pure compound **8** (Comp. **8**, 18 mg), compound **9** (Comp. **9**, 46 mg), and compound **10** (Comp. **10**, 17 mg). Finally, the 100% MeOH fr. (0.83 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH-HOAc (50:10:0.1, v/v) as an eluent, to yield four fractions; Fr. 1 (12 mg), Fr. 2 (98 mg), Fr. 3 (50 mg) and Fr. 4 (13 mg). Fr. 2 was further chromatographed on a Sephadex LH-20 column with MeOH to separate a pure compound **11** (Comp. **11**, 28 mg). The schematic procedure for extraction and isolation of eleven different phenolic compounds from mulberry cake is shown in Fig. 1.

Identification of phenolic compounds

UV-vis spectra of phenolic acids, flavonoids (in MeOH), and anthocyanins (0.1% HCl in MeOH) were determined with a photodiode array UV-vis spectrophotometer (Sinco, S-1100, Seoul, Korea). ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra of phenolic compounds were measured in each solvent (phenolic acids, CD_3OD; anthocyanins, 1% CF_3COOD in CD_3OD; flavonoids, DMSO-*d6*; 2-arylbenzofuran derivative, CD_3OD) on a spectrometer (Unity Plus 500, Varian, California, USA), and chemical shifts are given as δ value with tetramethylsilane (TMS) as an internal standard. Fast-atom bombardment mass spectrometry (FABMS) was recorded on a mass spectrometer (JEOL JMS-700, Tokyo, Japan, ion source, Xe atom beam; accelerating voltage, 10 kV) with glycerol as a mounting matrix.

DPPH, superoxide and hydroxyl radical scavenging activity

Radical scavenging activities of phenolic compounds against DPPH, superoxide and hydroxyl radical were determined (18,19). In the DPPH radical scavenging assay, each phenolic compound was added to 100 μM DPPH in methanol and incubated at 25°C. Their reactivity with DPPH was determined spectrophotometrically at 516 nm.

In the superoxide radical scavenging assay, the reaction mixture (3.0 mL) of 0.05 M Na_2CO_3 buffer (pH 10.2) containing 3 mM xanthine, 3 mM EDTA, BSA, 0.75 mM NBT, and 5.0 U/mL xanthine oxidase, and 6 mM $CuCl_2$ was incubated at 25°C for 20 min, and the formazan formed by the superoxide radical generated in the reaction mixture was measured spectrophotometrically at 560 nm.

In the hydroxyl radical scavenging assay, the reaction

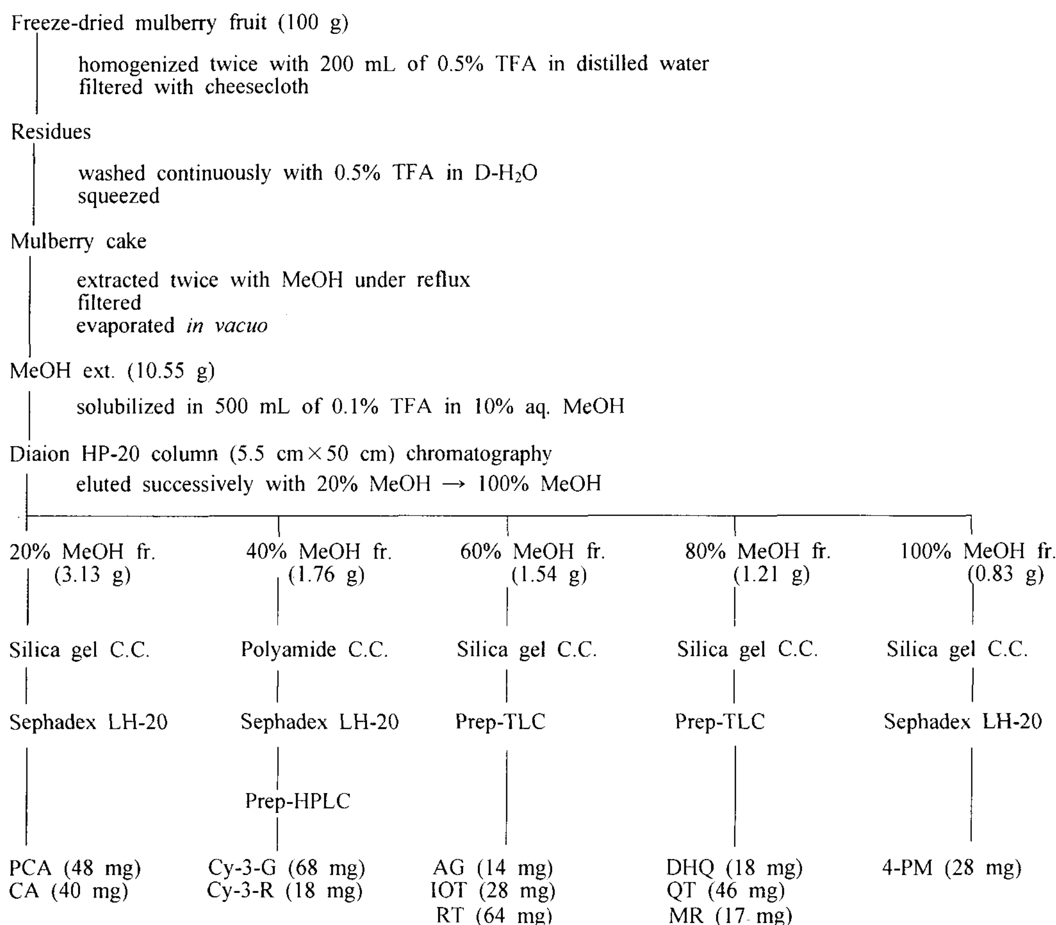

Fig. 1. Schematic procedure for the extraction, isolation and purification of phenolic compounds from mulberry cake.

mixture (2.0 mL) of 0.5 mg/mL of rat liver microsomal protein in 50 mM potassium phosphate buffer (pH 7.4), 30 mM H_2O_2, and 3.3 mM $FeSO_4$, was incubated at 37°C for 20 min. The thiobarbituric acid reactive substance (TBARS) level was determined after terminating the reaction by the addition of TBA reagent according to the method of Ohkawa et al. (20). IC_{50} values against three radical scavenging activities were determined by regression analysis at three different concentrations of the sample.

Statistical analysis

All experiments were performed in three replicates. Statistical analysis was performed using ANOVA with Duncan's multiple range test at $p<0.05$ (21).

RESULTS AND DISCUSSION

Identification of phenolic compounds

The MeOH extract from mulberry cake was successively subjected to a Diaion HP-20 (or polyamide), silica gel, Sephadex LH-20 and Prep-TLC (or Prep-HPLC), to give eleven phenolic compounds in pure states. Among them, two anthocyanins (Comp. **3**, cyanidin-3-*O*-β-D-glucoside; Comp. **4**, cyanidin-3-*O*-β-D-rutinoside), three flavonoid glycosides (Comp. **5**, astragalin; Comp. **6**, isoquercitrin; Comp. **7**, rutin), and three flavonoid aglycones (Comp. **8**, dihydroquercetin; Comp. **9**, quercetin; Comp. **10**, morin) had already been isolated and identified from mulberry fruits (16) and trees (22). The structures of two phenolic acids (Comp. **1** and Comp. **2**) and one 2-arylbenzofuran derivative (Comp. **11**) from mulberry fruits were first identified in this study.

Two phenolic compounds (Comp. **1** and **2**) yielded a protonated molecule $[M+H]^+$ at *m/z* 155 and 181, respectively, in the positive FAB-MS spectrum. ^{1}H-NMR (in CD_3OD) spectra of Comp. **1** showed signals characteristic of an aromatic ABX-type protons [6.79 (1H, *d*, *J*=8.0 Hz, H-5), 7.42 (1H, *dd*, *J*=1.5, 8.0 Hz, H-6), 7.48 (1H, *d*, *J*=1.5 Hz, H-2). In contrast, ^{1}H-NMR spectra of Comp. **2** exhibited aromatic ABX-type proton signals [6.77 (1H, *d*, *J*=8.5 Hz, H-5), 6.92 (1H, *dd*, *J*=1.5, 8.5 Hz, H-6), 7.03 (1H, *d*, *J*=1.5 Hz, H-2), and *trans* olefin proton signals [6.22 (1H, *d*, *J*=15.5 Hz, H-8)], 7.51 (1H, *d*, *J*=15.5 Hz, H-7)]. ^{13}C-NMR spectral data [Comp. **1**:

δ 171.17 (COOH), 151.25 (C-4), 146.0 (C-3), 123.83 (C-1), 122.67 (C-6), 117.78 (C-5), 115.73 (C-2); Comp. **2**: δ 171.41 (COOH), 149.43 (C-7), 146.85 (C-4), 146.74 (C-3), 127.95 (C-1), 122.82 (C-6), 116.53 (C-5), 116.05 (C-8), 115.10 (C-2)] of Comp. **1** and **2** coincided well with those of 3,4-dihydroxybenzoic acid and *trans*-3,4-dihydroxycinnamic acid isolated from plants (28). Thus, Comp. **1** and **2** were easily elucidated as 3,4-dihydroxybenzoic acid (procatechuic aid) and *trans*-3,4-dihydroxycinnamic acid (caffeic acid), respectively, which were first isolated from mulberry fruit, although two phenolic acids were already reported in other plants (23,24).

Meanwhile, one 2-arylbenzofuran derivative was elucidated by UV, IR, FABMS and NMR. The UV absorptions of Comp. **11** at λ_{max} 214, 295 (s), 320, 332 (s) nm were indicative of a 2-arylbenzofuran-type compound (25). Its IR spectrum suggested that Comp. **11** was a polyphenol-type compound without a carbonyl moiety. The positive FABMS of Comp. **11** gave a molecule peak at *m/z* 311 $[M+H]^+$, together with two significant fragment ion peaks at *m/z* 255 $[M^+-C_4H_7]$ and 241 $[M^+-C_5H_9]$, indicating the presence of a prenyl substituted 2-arylbenzofuran moiety. The ^{1}H-NMR spectrum showed that 4,6-disubstituted benzofuran moiety [aromatic AX-type proton signals at δ 7.20 (1H, *s*, H-7) and 6.86 (1H, d, *J*=1.0 Hz, H-5), and δ 6.88 (1H, *s*, H-3)], and 3′5′-disubstituted phenyl group [δ 6.74 (2H, *d*, *J*=2.5 Hz, H-2′ & H-6′) and 6.23 (1H, *t*, *J*=2.5 Hz, H-4′)], as well as a prenyl group [5.37 (1H, *t*, *J*=6.0 Hz, H-2″), 3.34 (2H, *brd*, *J*=6.0 Hz, H-1″), 1.76 & 1.74 (each 3H, *brs*)] were assignable to 4-prenyl substituted a arylbenzofuran moiety (25). The ^{13}C-NMR spectrum of Comp. **11** exhibited seventeen carbon signals, including 4-prenyl substituted 2-arylbenzofuran skeleton; 160.06 ($C_{3'}$ & $C_{5'}$), 155.87 (C_8), 155.71 (C_6), 154.77 (C_2), 134.15 ($C_{1'}$), 132.96 ($C_{3''}$), 126.32 (C_5), 124.56 (C_9), 122.89 ($C_{2''}$), 121.53 (C_4), 103.96 ($C_{2'}$ & $C_{6'}$), 103.47 ($C_{4'}$), 102.37 (C_3), 97.99 (C_7), 29.65 ($C_{5''}$), 26.12 ($C_{1''}$), 17.97 ($C_{4''}$). Based on these results, Comp. **11** was elucidated to be 4-prenylmoracin, which was first isolated from mulberry tree, even though its derivative was already isolated and identified from mulberry tree (25,26). The ^{1}H- & ^{13}C-NMR and FABMS spectra of 4-prenylmoracin are shown in Fig. 2~4.

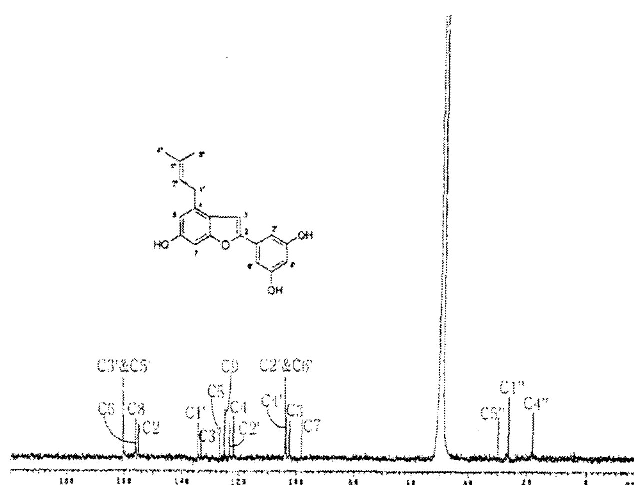

Fig. 2. ^{1}H-NMR spectrum of 4-prenylmoracin isolated from mulberry cake.

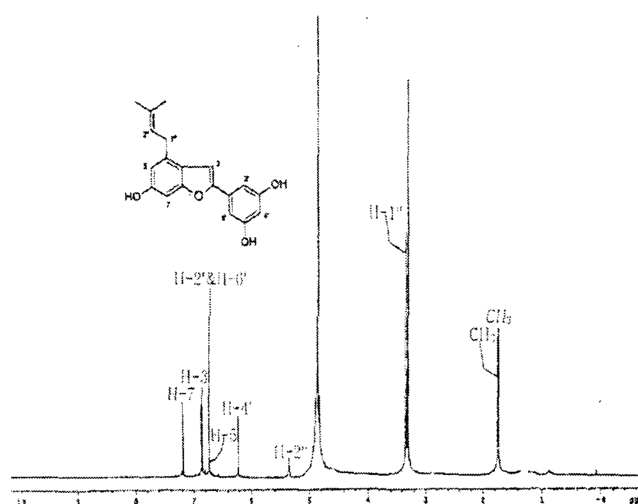

Fig. 3. ^{13}C-NMR spectrum of 4-prenylmoracin isolated from mulberry cake.

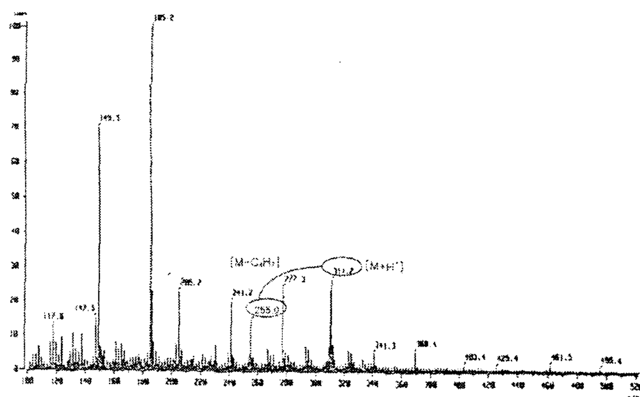

Fig. 4. FABMS spectrum of 4-prenylmoracin isolated from mulberry cake.

Radical scavenging activity of phenolic compounds

The radical scavenging activity of the eleven phenolic compounds isolated from mulberry cake was determined using three *in vitro* assays against the DPPH radical, superoxide anion generated enzymatically in a xanthine-xanthine oxidase system, and microsomal lipid peroxidation caused by the hydroxyl radical generated via the

Table 1. Radical scavenging activity (IC_{50}) of phenolic compounds isolated from mulberry cake

Compound	IC_{50} (μM)[1)]		
	DPPH	Superoxide	Hydroxyl
Procatechuic acid	38.25[c2)]	6.41[f]	49.45[a]
Caffeic acid	30.64[d]	1.84[g]	45.92[a]
Cyanidin 3-β-D-glucoside	>100	45.59[cd]	20.56[c]
Cyanidin 3-β-D-rutinoside	>100	56.84[c]	20.29[c]
Rutin	34.21[d]	14.72[e]	23.23[c]
Isoquercitrin	34.08[d]	14.57[e]	20.60[c]
Astragalin	43.87[b]	15.87[e]	37.42[b]
Quercetin	27.38[e]	50.95[c]	6.02[e]
Morin	28.32[e]	74.36[b]	20.98[c]
Dehydroquercetin	28.98[e]	5.23[f]	5.65[e]
4-Prenylmoracin	>100	82.53[a]	3.75[f]
BHT	53.82[a]	3.04[f]	10.57[d]

[1)] IC_{50} represents the concentration of a sample required for 50% inhibition of the DPPH, superoxide and hydroxyl radicals.
[2)] Values with different superscript letters are significantly different at $p<0.05$.

Fenton reaction. As shown in Table 1, most of phenolic compounds except two anthocyanins and 4-prenylmoracin scavenged DPPH radical more effectively than BHT ($p<0.05$). Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction (27). Therefore, phenolic compounds in mulberry cake acted as DPPH radical scavengers and could inhibit the lipid peroxidation.

Superoxide anions were generated enzymatically in a xanthine-xanthine oxidase system, and assayed by reduction of nitro blue tetrazolium. All phenolic compounds inhibited the generation of superoxide anion. Among them, caffeic acid was most effective in scavenging enzymatically generated superoxide anion with an IC_{50} value of 1.84 μM which was stronger than that of BHT ($p<0.05$). Moreover, dehydroquercetin and procatechuic acid showed potent superoxide radical scavenging activity with IC_{50} values of 5.23 and 6.41 μM which was weaker than that of BHT with an IC_{50} value of 3.04 μM ($p<0.05$). Other flavonoids and anthocyanins also exhibited considerable superoxide radical scavenging activities, with IC_{50} ranges of 15~75 μM, but 4-prenylmoracin showed the lowest activity. Finally, all phenolic constituents significantly inhibited microsomal lipid peroxidation induced by Fe(II)/H_2O_2. Among them, 4-prenylmoracin exhibited the most potent inhibitory activity with IC_{50} value of 3.75 μM, and its activity was stronger than that of BHT with an IC_{50} value of 10.57 μM ($p<0.05$). Dehydroquercetin and quercetin also showed strong activities with an IC_{50} values of 5.65 and 6.02 μM which were stronger than those of flavonoid glycosides, rutin (IC_{50}=23.23 μM), isoquercitrin (IC_{50}=20.60 μM) and astragalin (IC_{50}=37.42 μM). Other phenolic acids and anthocyanins exerted considerable hydroxyl radical scavenging activities which were weaker than that of BHT ($p<0.05$). Thus, phenolic compounds in mulberry cake, such as phenolic acids, flavonoids and 4-prenylmoracin, were shown to have considerable DPPH radical scavenging activity and to inhibit superoxide anion production in the xanthin-xanthine oxidase system and the hydroxyl radical-medicated lipid peroxidation of rat liver microsomes.

It is well established that lipid peroxidation is initiated by active oxygen species attacking unsaturated fatty acids and is propagated by a chain reaction cycle involving lipids, peroxy radicals and lipid hydroperoxides (27). Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxy radical (OH・), actively participate in the initiation of lipid peroxidation (28). Phenolic acids, anthocyanins and flavonoids are naturally occurring phenolics which are widely distributed in edible plants and foodstuffs derived from plants. Among them, hydroxycinnamic acids, cyanidins and flavonols are predominantly present in plant tissues. Phenolic compounds in plants have been reported to be strong radical scavengers against DPPH, superoxide and hydroxyl radicals (29-31). Several earlier works (31-33) speculated that the DPPH, superoxide and hydroxyl radical scavenging activities of phenolic acids increased with increasing of the number of phenolic hydroxyl groups in their compounds. Furthermore, free catechol groups at the 3′- and 4′-positions of flavonoids were reported to be essential for high superoxide and hydroxyl radical scavenging activity. Additionally, it was previously reported that there are some differences in the superoxide and hydroxyl radical-scavenging activities between flavonol aglycones and glycosides according to *in vitro* assay systems (33), and the radical scavenging activity of phenolic acids was relatively lower than that of flavonoids with benzopyron nuclei (34), although phenolic acids and flavonoids aglycones and their corresponding glycosides were equally efficient in scavenging DPPH free radical (31,33). Our data mostly supported these previous results, but the extent of radical scavenging activity was somewhat different between flavonoids and phenolic acids. Among phenolic compounds, caffeic acid potently scavenged superoxide, and dehydroquercetin greatly inhibited hydroxyl radical-mediated lipid peroxidation in rat liver microsomes. Meanwhile, 4-prenylmoracin having an arylbenzofuran moiety exhibited potently hydroxyl radical scavenging activity, even though its DPPH and superoxide radical scavenging activities were lower than those of other phenolic compounds. This result supported that the introduction of prenyl lipophilic substitution into

two hydroxyls of a moracin moiety increases its hydrophobicity, which is expected to improve its hydroxyl radical scavenging activity by enhancing its affinity to the microsomal membranes (35). This report first demonstrated that 2-arylbenzofuran derivatives can function as hydroxyl radical scavengers, although they have already been known to act as antidiuretic agents (22,26).

In conclusion, the methanol extract of mulberry cake prepared from mulberry fruits was previously found to have significant scavenging activity against DPPH, superoxide and hydroxyl radicals. The eleven phenolic compounds, including phenolic acids, anthocyanins, flavonoids and 2-arylbenzofuran derivative, were isolated and identified from mulberry cake. Most of phenolic compounds showed considerable scavenging activities, among which caffeic acid, dehydroquercetin and quercetin were most effective in inhibiting the generation of superoxide anion by the xanthine oxidase system and in preventing the microsonal lipid peroxidation induced by $Fe(II)/H_2O_2$. Other phenolic compounds also exhibited considerable radical scavenging activities, though less than that of BHT. These results suggest that several phenolic compounds could be mainly responsible for the strong radical scavenging activity of the methanol extract from mulberry cake, and furthermore that mulberry cake may be useful as a natural antioxidant which plays important physiological roles in the prevention of several active oxygen radical-mediated pathological conditions, such as cancer, inflammation, atherosclerosis, and aging. Further study is required to investigate the antioxidative activity of 2-arylbenzofuran derivatives *in vivo*.

ACKNOWLEDGMENTS

This research was funded by a special Grants Research Program (No. 102004-3) of the Problem-Oriented Technology Development Project for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea. We are grateful to Dr. Sung-Hong Kim and Ms. Yun-Kyeong Bae, Korea Basic Science Institute, Daegu, Korea, for NMR and FABMS measurements.

REFERENCES

1. Frei B. 1994. Nonenzymatic antioxidant defense systems. In *Natural antioxidants in human health and disease*. Briviba K, Sies H, eds. Academic Press, London. p 107-118.
2. Beckman KB, Ames BN. 1997. Oxidant, antioxidant, and aging. In *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. Scandalios JG, ed. Cold Spring Harbor Laboratory Press, New York. p 201-246.
3. Okuda T, Yoshida T, Hatano T. 1993. Antioxidant phenolics in oriental medicine. In *Active Oxygen, Lipid Peroxides, and Antioxidants*. Yagi K, ed. CRC Press, New York. p 333-346.
4. Schuler P. 1990. Natural antioxidants exploited commercially. In *Food Antioxidants*. Hudson BJF, ed. Elsevier, London. p 99-121.
5. Lee SI. 1981. Mori Fructus. In *Bonchohak*. Suseowon, Seoul. p 136-137.
6. Park JH. 2002. Mori Fructus. In *The encyclopedia of chinese crude drugs*. Shinilsangsa, Seoul. p 421-422.
7. Kim TY, Kwon YB. 1996. A study on the antidiabetic effect of mulberry fruits. *Kor J Seri Sci* 38: 100-107.
8. Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS, Ryu KS. 2001. Polyhydoxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). *J Agric Food Chem* 49: 4208-4213.
9. Park JC, Choi JS, Choi JW. 1995. Effects of the fractions from the leaves, fruits, stems and roots of *Cudrania tricuspidata* and flavonoids on lipid peroxidation. *Kor J Pharmacogn* 26: 377-384.
10. Kim SY, Park KJ, Lee WC. 1998. Antiinflammatory and antioxidative effects of *Morus* spp. fruit extract. *Kor J Med Crop Sci* 6: 204-209.
11. Kim HJ, Cha JY, Choi ML, Cho YS. 2000. Antioxidative activities by water-souble extracts of *Morus alba* and *Cudrania tricuspidata*. *J Kor Soc Agric Chem Biotechnol* 43: 148-152.
12. Kim HB, Kim SY, Ryu KS, Lee WC, Moon JY. 2001. Effect of methanol extract from mulberry fruit on the lipid metabolism and liver function in cholesterol-induced hyperlipidemia rats. *Kor J Seri Sci* 43: 104-108.
13. Kim HB, Kim AJ, Kim SY. 2003. The analysis of functional materials in mulberry fruit and food product development trends. *Food Sci Indus* 36: 49-60.
14. Gerasopoulos D, Stavroulakis G. 1997. Quality characteristics of four mulberry (*Morus spp*) cultivars in the area of Chania, Greece. *J Sci Food Agric* 73: 261-264.
15. Lee HW, Shin DH, Lee WC. 1998. Morphological and chemical characteristics of mulberry (*Morus*) fruit with varieties. *Kor J Seric Sci* 40: 1-7.
16. Lee JY, Moon SO, Kwon YJ, Rhee SJ, Choi SW. 2004. Identification and quantification of anthocyanins and flavonoids in mulberry (*Morus* spp.) cultivars. *Food Sci Biotechnol* 13: 176-184.
17. Kim IS, Lee JY, Rhee SJ, Yun KS, Choi SW. 2004. Preparation of mulberry juice using minimally process technology. *J Kor Soc Food Sci Technol* 36: 321-328.
18. Kwon YJ, Rhee SJ, Chu JW, Choi SW. 2005. Comparison of radical scavenging activity of extracts of mulberry juice and cake prepared from mulberry (*Morus* spp.) fruit. *J Food Sci Nutr* 10: 111-117.
19. Kang GH, Chang EJ, Choi SW. 1998. Antioxidative activity of phenolic compounds in roasted safflower (*Carthamus tinctorius* L.) seeds. *J Food Sci Nutr* 4: 134-139.
20. Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95: 351-358.
21. SAS Institute, Inc. 1985. *SAS User's Guide: Statistics*. Cary, NC.
22. Basnet P, Kadota S, Terashima S, Shimizu M, Namba T. 1993. Two new 2-arylbenzofuran derivatives from hypoglycemic activity-bearing fractions of *Morus insignis*. *Chem Pharm Bull* 41: 1238-1243.
23. Pouchert CJ, Behnke J. 1993. Aromatic carboxylic acids. In *The Aldrich Library of ^{13}C and ^{1}HFT NMR Spectra*. Aldrich Chemical Co. Inc., USA. Vol 2, p 1058, 1116.

24. Cai Y, Luo O, Sun M, Corke H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci* 74: 2157-2184.
25. Takasugi M, Nagao S, Masamune T. 1982. Structure of moracin A and D, new phytoalexins from diseased mulberry. *Tetrahedron Letters* 9: 797-798.
26. Fukai T, Hano Y, Hirakura K, Nomura T, Uzawa J, Fukushima K. 1984. Structures of mulberrofuran F and G, two natural hypotensive diels-alder type adducts from the cultivated mulberry tree (*Morus Lhou* (Ser.) Koidz). *Heterocycles* 22: 473-477.
27. Witting LA. 1980. Vitamin E and lipid antioxidants in free-radical-initiated reactions. In *Free Radicals in Biology*. Pryor WA, ed. Academic Press, New York. Vol 4, p 295-319.
28. Mayumi T, Schiller HJ, Bulkley GB. 1993. Pharmaceutical intervention for the prevention of post-ischemic reperfusion injury. In *Free Radicals: From Basic Science to Medicine*. Poli G, Albamo E, Dianzni U, eds. Birkhauser Verlag, Switzerland. p 438-457.
29. Bors W, Heller W, Michel C, Saran M. 1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. In *Methods in Enzymology*. Packer L, Glazer AN, eds. Academic Press, New York. Vol 186, p 343-355.
30. Wang H, Cao G, Prior RL. 1997. Oxygen radical absorbing capacity of anthocyanins. *J Agric Food Chem* 45: 304-309.
31. Wang P, Kang J, Zheng R, Yang Z, Lu J, Gao J, Jia Z. 1996. Scavenging effects of phenylpropanoid glycosides from *Pedicularis* on superoxide anion and hydroxyl radical by the spin trapping method. *Biochem Pharmacol* 51: 687-691.
32. Mora A, Paya M, Rios JL, Alcaraz MJ. 1990. Structure-activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochem Pharmacol* 40: 793-797.
33. Haraguchi H. 2001. Antioxidative plant constituents. In *Bioactive Compounds from Natural Sources.* Corrado T, ed. Taylor & Francis Inc., New York. p 339-377.
34. Das NP, Ramanathan L. 1992. Studies on flavonoids and related compounds as antioxidants in food. In *Lipid-Souble Antioxidants: Biochemistry and Clinical Applications*. Ong ASH, Packer L, eds. Birkhauser Verlag, Switzerland. p 295-306.
35. Kagan VE, Quinn PJ. 1988. The interaction of α-tocopherol and homologues with shorter hydrocarbon chains with phospholipid bilayer dispersions. A fluorescence probe study. *Eur J Biochem* 171: 661-667.

(Received October 24, 2005; Accepted December 2, 2005)