Antioxidative Flavonoids from the Leaves of *Morus alba*

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(Received September 8, 1998)

Nine flavonoids (1-9) were isolated from the leaves of *Morus alba* (Moraceae). The structures of compounds were determined to be kaempferol-3-O-D-glucopyranoside (astragalin, 1), kaempferol-3-O-(6"-O-acetyl)-β-D-glucopyranoside (2), quercetin-3-O-(6"-O-acetyl)-β-D-glucopyranoside (3), quercetin-3-O-β-D-glucopyranoside (4), kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (5), quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (rutin, 6), quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (7), quercetin-3,7-di-O-β-D-glucopyranoside (8) and quercetin (9) on the basis of spectroscopic and chemical studies. Compounds 7 and 9 exhibited significant radical scavenging effect on 1,1-diphenyl-2-picryl-hydrazyl radical.

**Key words**: *Morus alba*, Moraceae, DPPH radical, Antioxidative activity

INTRODUCTION

Mulberry (*Morus alba* L.) leaves containing many nutritional components are the best feed for silkworms. It was reported that the extracts from mulberry leaves showed a potent antihyperglycemic activity in diabetic mice (Chen et al., 1989; Chen et al., 1995). N-containing sugars (Asano et al., 1994; Yagi et al., 1976), plant hormone (Takimoto et al., 1967), and moracetin (Naitoh, 1969) have been also isolated from mulberry leaves. Many phenolic compounds have been identified from the root barks of mulberry tree (Nomura and Fukai, 1981). *M. alba* contained rutin (Naitoh, 1968), isoquercitrin (Onogi et al., 1993) and astragalin (Onogi et al., 1993). However, antioxidative activities of flavonoids of *M. alba* have not been reported so far. We isolated nine flavonoids 1-9 from the 85% methanol extract of the leaves of *M. alba*. Compounds 5, 7 and 8 have been isolated from the leaves of the mulberry tree for the first time. This paper reports the isolation, structure elucidation, and the free radical scavenging property of compounds 1-9.

MATERIALS AND METHODS

**Instrumentation and general techniques**

UV spectra were obtained on a Unical UV/VIS spectrophotometer. IR spectra were recorded with a Jasco FT/IR 5300 spectrometer, ¹H- and ¹³C-NMR spectra were run on a Bruker AMX 500 spectrometer. FABMS spectra were measured on a VG 70-VSEQ mass spectrometer with a direct inlet system using PEG600/glycerol as a matrix. TLC and column chromatography was carried out on Merck precoated silica gel F254 plates and Si gel 60 (Merck, 70~230 mesh) or sephadex LH 20 (Sigma, 25~100 μm). All other chemicals and solvent were analytical grade and used without further purification.

**Plant materials**

Mulberry (*M. alba*) leaves were collected at Suwon, Kyunggi Province, Korea in June, 1997. The voucher specimen is deposited in the herbarium of the Department of National Sericulture and Entomology, NIAST, RDA. The leaves were harvested, cleaned, freeze-dried and ground into fine powder in a mill (Tecator cemotec 1090 sample mill, Hoganas, Sweden). The material that passed through an 80-mesh sieve was retained, sealed in a glass bottle and stored at 4°C until use.

**Extraction and isolation**

Dry powdered leaves of *M. alba* (2 kg) were extracted with a mixture of water and methanol (15:85) in a sonicator (5×5 l, 25°C) for 1 hour and then concentrated under vacuum. The resulting MeOH extract (287 g) was suspended in water (200 ml), and...
was partitioned with CH₂Cl₂ (500 ml×5) and BuOH (400 ml×5), successively, to give CH₂Cl₂ (53 g), BuOH (43 g), and H₂O (166 g) fractions. The BuOH extract (43 g) was subjected to Diaion HP-20 column chromatography with H₂O (3500 ml), 50% EtOH (2000 ml), and EtOH (2000 ml) respectively. The 50% EtOH eluate (18 g) was chromatographed on a silica gel column (55×5 cm) eluting with CH₂Cl₂-MeOH-H₂O (80:10:0, 2500 ml); 60:10:0 (2100 ml); 40:10:1 (1530 ml); 50:15:2 (2100 ml); 70:30:5 (3150 ml); 0:1:0 (3000 ml) to give 12 fractions (A-L). Fraction C was then subjected to Sephadex LH-20 chromatography with MeOH to obtain two subfractions C₁ and C₂. C₁ was further chromatographed on silica gel to obtain compound 1 (105 mg, 0.005%). A mixture which was subjected to Sephadex LH-20 column chromatography eluting with H₂O-MeOH (1:1, 250 ml) to give compound 2 (13 mg, 0.001%). Fraction D was subjected to LH-20 chromatography with H₂O-MeOH (3:7, 300 ml) to give a mixture with compounds D-a and D-b which was chromatographed again on a silica gel column eluting with EtOAc-MeOH-H₂O (25:1:1, 1000 ml) to obtain a pure compound 3 (20 mg, 0.001%). Fraction F was subjected to Sephadex LH-20 chromatography with gradient H₂O-MeOH (2:8, 400 ml) and produced 280 mg, a yield of 0.028%, of compound 4. Compound 1 (astragalin); pale yellow needles, mp. 231-234°C; IR νmax (cm⁻¹): 3439 (OH), 1655 (C=O), 1604, 1498 (C=C), 1062, 1016 (glycosidic C-O); UV (MeOH) λmax: 214, 267, 302 (sh), 349 nm; FABMS (m/z) 449 [MH]+; ¹H-NMR (DMSO-d₆, 500 MHz, ppm): δ 6.19 (1H, d, J=2.0 Hz, H-6), 6.64 (1H, d, J=2.0 Hz, H-8), 8.04 (2H, d, J=8.8 Hz, H'2, 6'), 6.88 (2H, d, J=8.8 Hz, H-3', 5'), 5.45 (1H, d, J=7.5 Hz, H-1''); ¹³C-NMR (DMSO-d₆, 500 MHz, ppm): δ 156.2 (C-2), 133.2 (C-3), 177.3 (C-4), 159.9 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.5 (C-9), 103.9 (C-10), 120.8 (C-1'), 1130.8 (C-2'), 115.1 (C-3'), 161.2 (C-4'), 115.1 (C-5'), 130.8 (C-6'), 100.9 (C-1''), 74.2 (C-2''), 76.4 (C-3''), 69.9 (C-4''), 77.4 (C-5''), 60.8 (C-6''). Acid hydrolysis of 1 gave kaempferol and glucose.

Compound 2 (kaempferol-3-O(6'-O-acetyl)-β-D-glucopyranoside); yellow needles, mp 258-263°C; IR νmax (cm⁻¹): 3412 (OH), 1721 (-COCH₃), 1655 (C=O), 1609, 1512 (aromatic C=C), 1209 (ester), 1180, 1086 (glycosidic C-O); UV (MeOH) λmax: 208, 266, 320, 350 nm; FABMS (m/z) 491 [MH]+; ¹H-NMR (DMSO-d₆, 500 MHz, ppm): δ 6.20 (1H, d, J=2.0 Hz, H-6), 6.44 (1H, d, J=2.0 Hz, H-8), 7.98 (2H, dd, J=8.8, 2.0 Hz, H-2', 6'), 6.87 (2H, d, J=8.8 Hz, H-3', 5'), 15.12 (brs, 5-OH), 5.35 (1H, d, J=7.4 Hz, H-1''), 10.83, 10.13 (each brs, 4', 7-OH) 1.73 (3H, s, -COCH₃); ¹³C-NMR (DMSO-d₆): δ 158.4 (C-2), 134.7 (C-3), 178.4 (C-4), 161.6 (C-5), 99.43 (C-6), 164.9 (C-7), 94.5 (C-8), 157.4 (C-9), 104.7 (C-10), 122.4 (C-1''), 115.7 (C-2''), 144.8 (C-3') 148.9 (C-4''), 177.0 (C-5'), 122.9 (C-6''), 101.10 (C-1'), 74.0 (C-2'), 76.1 (C-3'), 69.7 (C-4''), 73.8 (C-5''), 62.8 (C-6''), 169.7, 20.1 (-COCH₃). Acid hydrolysis of 2 gave kaempferol and glucose.

Compound 3 (quercetin-3-O(6'-O-acetyl)-β-D-glucopyranoside); yellow needles, mp 217-219°C; IR νmax (cm⁻¹): 3310 (OH), 1665 (C=O), 1607 (aromatic), 1086 (C-O); UV (MeOH) λmax: 208, 257, 267 (sh), 359 nm; FABMS (m/z) 507 [MH]+; ¹H-NMR (DMSO-d₆, 500 MHz, ppm): δ 6.20 (1H, s, H-6), 6.40 (1H, s, H-8), 7.49 (1H, s, H-2'), 6.82 (1H, d, J=8.8 Hz, H-5'), 7.50 (1H, d, J=8.8 Hz, H-6'), 5.31 (1H, d, J=7.4 Hz, H-1''), 1.68 (3H, s, -COCH₃); ¹³C-NMR (DMSO-d₆, 125 MHz, ppm): δ 156.3 (C-2), 133.2 (C-3), 177.3 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.4 (C-9), 103.8 (C-10), 121.5 (C-1''), 115.1 (C-2''), 144.8 (C-3''), 148.4 (C-4''), 116.1 (C-5'), 121.0 (C-6''), 100.9 (C-1''), 74.2 (C-2''), 76.4 (C-3''), 69.9 (C-4''), 73.9 (C-5''), 62.8 (C-6''), 169.8, 20.0 (-COCH₃). Acid hydrolysis of 3 gave quercetin and glucose.

Compound 4 (quercetin-3-O-β-D-glucoside); yellow needles, mp 238-241°C; IR νmax (cm⁻¹): 3310 (OH), 1665 (C=O), 1607 (aromatic), 1086 (C-O); UV (MeOH) λmax: 208, 257, 267 (sh), 359 nm; FABMS (m/z) 465 [MH]+; ¹H-NMR (DMSO-d₆, 500 MHz, ppm): δ 6.20 (1H, s, H-6), 6.40 (1H, s, H-8), 7.49 (1H, s, H-2'), 6.82 (1H, d, J=8.8 Hz, H-5'), 7.50 (1H, d, J=8.8 Hz, H-6'), 5.31 (1H, d, J=7.4 Hz, H-1''), 1.68 (3H, s, -COCH₃); ¹³C-NMR (DMSO-d₆, 125 MHz, ppm): δ 156.1 (C-2), 133.2 (C-3), 177.3 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.4 (C-9), 103.8 (C-10), 121.5 (C-1''), 115.1 (C-2''), 144.8 (C-3''), 148.4 (C-4''), 116.1 (C-5'), 121.0 (C-6''), 100.9 (C-1''), 74.2 (C-2''), 76.4 (C-3''), 69.9 (C-4''), 73.9 (C-5''), 62.8 (C-6''), 169.8, 20.0 (-COCH₃). On acid hydrolysis, 4 gave quercitin and glucose.

Compound 5 (kaempferol-3-O-α-L-rhamnopyranosyl-1-β-D-glucopyranoside); yellow needles, mp 215-220°C; IR νmax (cm⁻¹): 3310 (OH), 1665 (C=O); UV (MeOH) λmax: 208, 266, 349, 359 nm; FABMS (m/z)
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595 [MH]+; 1H-NMR (DMSO-d6, 500 MHz, ppm): δ 6.20 (1H, d, J=2.0 Hz, H-6), 6.40 (1H, d, J=2.0 Hz, H-8), 7.98 (2H, dd, J=8.8, 2.0 Hz, H-2', 6'), 6.86 (2H, dd, J=8.8, 2.0 Hz, H-3', 5'), 0.98 (3H, d, J=4 Hz, -CH3 of rhamnose), 5.31 (1H, d, J=7.5 Hz, glc-1); 13C-NMR (DMSO-d6, 125 MHz, ppm): δ 156.8 (C-2), 133.2 (C-3), 177.3 (C-4), 159.8 (C-5), 98.7 (C-6), 164.1 (C-7), 93.7 (C-8), 103.9 (C-9), 156.4 (C-9), 103.9 (C-10), 120.8 (C-1'), 130.8 (C-2'), 115.0 (C-3'), 115.0(C-5'), 130.8 (C-6'), 100.7 (C-1'), 74.1 (C-2'), 76.3 (C-3'), 69.9 (C-4'), 75.7 (C-5'), 66.8 (C-6'), 101.3 (C-1'), 70.5 (C-2'), 70.3 (C-3'), 71.8 (C-4'), 68.2 (C-5'), 17.7 (C-6'). On acid hydrolysis, 5 gave kaempferol, rhamnose and glucose.

Compound 6 (quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside); yellow needles, mp 186°C; IR νmax cm⁻¹: 3300 (OH), 1650 (C=O); UV (MeOH) λmax: 209, 256, 267(sh), 357 nm; FABMS (m/z) 627 [MH]+; 1H-NMR (DMSO-d6, 500 MHz, ppm): δ 6.19 (1H, s, H-6), 6.40 (1H, s, H-8), 7.54 (1H, d, J=2.0 Hz, H-2'), 6.68 (1H, d, J=8.8 Hz, H-5'), 7.59 (1H, d, J=8.0, 2.0 Hz, H-6'), 5.69 (1H, d, J=7.6, H-1'), 4.60 ppm (1H, d, J=7.7 Hz). On acid hydrolysis, 7 gave quercetin and glucose.

Compound 7 (quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside); yellow powder, mp 224-228°C; IR νmax cm⁻¹: 3310 (OH), 1665 (C=O); UV (MeOH) λmax: 209, 256, 370 nm, UV (NaOAc) λmax: 259, 383 nm, UV (AlCl3) λmax: 275, 434 nm, UV (AlCl3+HCl) λmax: 270, 299 (sh) nm; FABMS (m/z) 627 [MH]+; 1H-NMR (DMSO-d6, 125 MHz, ppm): δ: 158.4 (C-2), 134.7 (C-3), 178.4 (C-4), 161.6 (C-5), 99.4 (C-6), 164.9 (C-7), 94.5 (C-8), 157.4 (C-9), 104.7 (C-10), 122.4 (C-1'), 115.7 (C-2'), 144.8 (C-3'), 148.9 (C-4'), 117.0(C-5'), 122.9 (C-6'), 101.4 (C-1'), 74.6 (C-2'), 76.9 (C-3'), 70.0 (C-4'), 75.9 (C-5'), 68.8 (C-6'), 103.7 (C-1''), 71.2 (C-2''), 70.8 (C-3''), 72.7 (C-4''), 67.5 (C-5''), 17.4 (C-6''). On acid hydrolysis, 6 gave quercetin and glucose.

Compound 8 (quercetin-3,7-di-O-β-D-glucopyranoside); yellow needles; IR νmax cm⁻¹: 3350 (OH), 1685 (C=O); UV (MeOH) λmax: 209, 256, 1650 (C=O); UV (NaOAc) λmax: 259, 383 nm, UV (AlCl3+H3BO4) λmax: 261, 383 nm, UV (AlCl3) λmax: 275, 434 nm, UV (AlCl3+HCl) λmax: 270, 299 (sh) nm; FABMS (m/z) 627 [MH]+; 1H-NMR (DMSO-d6, 500 MHz, ppm): δ: 6.43 (1H, d, J=2.0 Hz, H-6), 6.75 (1H, d, J=2.0 Hz, H-8), 7.59 (1H, d, J=2.0 Hz, H-2'), 6.65 (1H, d, J=8.5 Hz, H-5'), 7.58 (1H, dd, J=8.5, 2.0 Hz, H-6'), 5.48 (1H, d, J=7.3 Hz, H-1'), 3.08 (1H, d, J=7.3 Hz, H-1''); 13C-NMR (DMSO-d6, 125 MHz, ppm): δ: 156.0 (C-2), 133.4 (C-3), 177.6 (C-4), 160.7 (C-5), 99.3 (C-6), 162.7 (C-7), 94.4 (C-8), 156.8 (C-9), 105.6 (C-10), 121.4 (C-1'), 115.2 (C-2'), 144.4 (C-3'), 148.8 (C-4'), 116.3 (C-5'), 120.4 (C-6'), 100.7 (C-1''), 74.2 (C-2''), 76.4 (C-3''), 69.9 (C-4''), 77.5 (C-5''), 60.8 (C-6''), 99.7 (C-1''''), 73.0 (C-2''''), 76.4 (C-3''''), 69.6 (C-4''''), 77.2 (C-5''''), 60.6 (C-6''''). On acid hydrolysis, 8 gave quercetin and glucose.

Compound 9 (quercetin); yellow needles, mp 311-313°C (dec.); IR νmax cm⁻¹: 3350 (OH), 1685 (C=O); UV (MeOH) λmax: 255, 370 nm, UV (MeOH) λmax: 321; FABMS (m/z) 303 [MH]+; 1H-NMR (DMSO-d6, 500 MHz, ppm): δ 6.15 (1H, s, H-6), 6.35 (1H, s, H-8), 7.47 (1H, d, J=2.0 Hz, H-2'), 6.81 (1H, d, J=8.5 Hz, H-5'), 7.40 (1H, d, J=8.5 Hz, H-6'); 13C-NMR (DMSO-d6, 125 MHz, ppm): δ: 158.4 (C-2), 134.7 (C-3), 178.4 (C-4), 161.6 (C-5), 99.4 (C-6), 164.9 (C-7), 94.5 (C-8), 157.4 (C-9), 104.7 (C-10), 122.4 (C-1'), 115.7 (C-2'), 144.8 (C-3'), 148.9 (C-4'), 117.0(C-5'), 122.9 (C-6'), 101.4 (C-1'), 74.6 (C-2'), 76.9 (C-3'), 70.0 (C-4'), 75.9 (C-5'), 68.8 (C-6'), 103.7 (C-1''), 71.2 (C-2''), 70.8 (C-3''), 72.7 (C-4''), 67.5 (C-5''), 17.4 (C-6''). On acid hydrolysis, 6 gave quercetin, rhamnose and glucose.

Fig. 1. The structures of compounds 1-9
ppm): δ 146.9 (C-2), 135.8 (C-3), 175.9 (C-4), 160.8 (C-5), 98.3 (C-6), 164.0 (C-7), 93.5 (C-8), 156.2 (C-9), 103.1 (C-10), 122.1 (C-1'), 115.2 (C-2'), 145.1 (C-3'), 147.7 (C-4'), 115.7 (C-5'), 120.1 (C-6').

Acid hydrolysis of compounds

Three mgs. of each compound was dissolved in 2 ml of 2 N HCl-MeOH (1:1) in a 10 ml round bottomed flask and heated at 100°C for 1 h. To isolate the sugar and aglycons for further analysis, the reaction mixture was evaporated to half of original volumes to remove the MeOH, and then extracted several times with EtOAc by shaking vigorously in a test tube. In each case, the aglycon was fractionated into the EtOAc layer and the sugar into H₂O layer. The aglycone portion was analyzed by analytical HPLC with an ODS column (CH₃CN-H₂O, 1:1, 250x4.60 mm) and the sugar portion by analytical HPLC with a carbohydrate column (CH₃CN-H₂O, 4:1, 300x3.90 mm). The retention times were compared with those of authentic samples.

Testing for antioxidant activity

MeOH solutions (4 ml) of flavonoids at various concentrations (1-14 µg/ml) were added to a solution of DPPH in MeOH (1.5x10⁻⁴ M, 1 ml) and the reaction mixture were shaken vigorously. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm (Yoshida et al., 1989). And the radical scavenging activity of each flavonoid was expressed by the ratio of the lowering of the absorption of DPPH (%) relative to the absorption (100%) of the DPPH solution in the absence of flavonoids. The mean values were obtained from triplicate experiments.

RESULTS AND DISCUSSION

The investigation of 85% methanol extract of the leaves of M. alba yielded nine flavonoids. Compounds 5, 7, and 8 were reported from M. alba for the first time.

The structures of compound 1-4, 6 and 9 were determined by comparison of their mps, UV, and NMR spectral data with those reported in the literature; Compound 1 was determined as astragalin (Kim et al., 1995), compound 2 as kaempferol-3-O-(6'-O-acetyl)-β-D-glucopyranoside (Do et al., 1992), compound 3 as quercetin-3-O-(6'-O-acetyl)-β-D-glucopyranoside (Onogi et al., 1993), compound 4 as isoquercitrin (Kang and Woo, 1984), compound 6 as rutin (Shin et al., 1995), and compound 9 as quercetin (Kim et al., 1995), respectively.

The ¹H- and ¹³C- NMR spectra of compound 5 were similar to those of compound 1, except for the presence of significant signals of rhamnose [¹H: 5.33 ppm (1H, brs, H-1), 0.98 ppm (3H, J=6.2 Hz H-6), ¹³C:101.3 (C-1) and 17.7 ppm (C-6)]. Compared to compound 1, C-6 signal of compound 5 shifted downfield from 60.8 ppm in compound 1 to 66.8 ppm. It was shown that rhamnose links to C-5 of glucose. From the chemical shifts and the coupling constant of the anomeric proton of rhamnose, the glycosidic linkage of rhamnose is α type. From these data, compound 5 was identified as kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (Takagi et al., 1981). Compound 7 was isolated in the form of yellow powder. ¹H- and ¹³C-NMR spectra were similar to those of compound 4, isoquercitrin except for the presence of an additional glucose signals [¹H: 4.60 ppm (1H, d, J=7.7 Hz)]. The type of glycosidic linkage was determined to be β from the chemical shift and the coupling constant of the anomeric proton of glucose. In the ¹³C-NMR spectrum, the C-6 signal shifted downfield compared to isoquercitrin. Thus, the structure of compound 7 was established as quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (Nohara et al., 1982). The orthodihydroxyl group in the B-ring of compound 8 was confirmed on the basis of band I in the AICI₃ and AICI₅/HCl spectrum as well as the NaOAc/ H₂BO₃ spectrum. ¹H NMR spectrum of compound 8 was similar to that isoquercitrin except for the presence of an additional glucose unit [¹H: 5.08 (d, J=7.3) for anomeric proton signal]. This showed that compound 8 was a monoglucose of isoquercitrin. Comparing with isoquercitrin, H-6 and H-8 signals of compound 8 were shifted downfield to 6.43 ppm and 6.75 ppm, respectively. The glycosidation site was also deduced from ¹³C-NMR data (Harborne and Mabry, 1982). Only the C-3 and C-7 signals were shifted upfield by 2.4 and 1.3 ppm respectively in comparison with quercetin. These changes confirmed that the quercetin was substituted at C-3 and C-7. The downfield shifts of C-2, C-4, C-6 and C-8 signals also supported this assignment.

Table 1. Radical scavenging effects of compounds 1-9 on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical

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<tr>
<td>compound 9</td>
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</tbody>
</table>

*Each compound was examined with four concentration in triplicate.

*ED₅₀ value represents the concentration of a compound required for 50% decreases of DPPH radicals.
coupling constant ($\nu=7.3$ Hz) between H-1 glucose and H-2 glucose indicated that the linked sugars at both site were $\beta$ configuration. The pyranose form of sugar was established on the basis of $^{13}$C-NMR spectral data (Markham et al., 1978). From the above data, compound 8 was determined to be quercetin-3, 7-di-O-$\beta$-D-glucopyranoside (Kodama et al., 1990).

Damage caused by the toxic effects of OH$^-$ radicals is often decreased by radical scavengers such as phenolic compounds (Cederbaum and Cohen, 1985; Halliwell and Gutteridge, 1984). The flavonoids are also known to act as strong superoxide radicals (O$_2^-$) scavengers and singlet oxygen (O$_2$) quenchers (Husain, 1987). To examine the radical scavenging effects of flavonoids, nine flavonoids isolated from leaves of Morus alba L. were tested by the $\alpha,\alpha$-diphenyl-$\beta$-picrylhydrazyl (DPPH) radical. (Table I) The radical scavenging activity of the flavonoids isolated decreases in the order: Compound 9 > Compound 7 > Compound 3 > Compound 8 > Compound 6 > Compound 4. But compounds 1, 2 and 5 showed scarcely any effect. The compound 9 exhibited higher activity than BHA, positive standard.

ACKNOWLEDGEMENTS

The authors thank Eun Jung Bang from Korea Basic Science Institute for the NMR and FABMS spectra.

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