Arch Pharm Res Vol 25, No 3, 313-319, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Antioxidative Flavonoids from Leaves of *Carthamus tinctorius*

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(Received March 4, 2002)

A total of eight flavonoids (**1-8**), including a novel quercetin-7-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside (**6**) and seven known flavonoids, luteolin (**1**), quercetin (**2**), luteolin 7-*O*-β-D-glucopyranoside (**3**), luteolin-7-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside (**4**) quercetin 7-*O*-β-D-glucopyranoside (**5**), acacetin 7-*O*-β-D-glucuronide (**7**) and apigenin-6-*C*-β-D-glucopyrano syl-8-*C*-β-D-glucopyranoside (**8**), have been isolated from the leaves of the safflower (*Carthamus tinctorius* L.) and identified on the basis of spectroscopic and chemical studies. The antioxidative activity of these flavonoids was evaluated against 2-deoxyribose degradation and rat liver microsomal lipid peroxidation induced by hydroxyl radicals generated via a Fenton-type reaction. Among these flavonoids, luteolin-acetyl-glucoside (**4**) and quercetin-acetyl-glucoside (**6**) showed potent antioxidative activities against 2-deoxyribose degradation and lipid peroxidation in rat liver microsomes. Luteolin (**1**), quercetin (**2**), and their corresponding glycosides (**3** & **5**) also exhibited strong antioxidative activity, while acacetin glucuronide (**7**) and apigenin-6,8-di-*C*-glucoside (**8**) were relatively less active.

Key words: *Carthamus tinctorius*, Compositae, Flavonoids, 2-Deoxyribose degradation, microsomal lipid peroxidation, Antioxidative activity

INTRODUCTION

Naturally occurring flavonoids have attracted much attention as dietary antioxidants capable of inhibiting oxygen radical-mediated lipid peroxidation which is reportedly associated with several pathological diseases (DiSilvestro, 2001). For this reason, an extensive search for novel antioxidative flavonoids from plant sources has been undertaken.

Safflower(*Carthamus tinctorius* L. Compositae) is widely distributed in eastern and western Asia. The flower of safflower is used in folk medicine as an analgesic, antithrombotic and antihypertensive crude drug, as well as a source of natural colorants (Han, 1988). Additionally, safflower seeds rich in α-linoleic acid are commonly consumed as vegetable oil in the USA and Europe, and used clinically for the treatment of cataclasis, osteoporosis and rheumatoid arthritis in Korea (Kim, 1992). Recent studies revealed that safflower seeds have been shown to improve lipid metabolism in high fat and cholesterol-fed rats (Kim *et al.*, 1999; Cho *et al.*, 2001), and to protect against bone fracture and loss (Kim *et al.*, 1998; Chung *et al.*, 1999; Kim *et al.*, 2001). Several phenolic compounds, including serotonins, lignans and flavonoids with antioxidative and physiological actions have been isolated and identified from safflower seeds (Zhang *et al.*, 1997; Kawashima *et al.*, 1998; Roh *et al.*, 1999; Kang *et al.*, 1999; Takii *et al.*, 1999; Bae *et al.*, 2001) and flowers (Baek *et al.*, 1998). Thus, although many studies have reported on the chemistry and phamacological activities of the safflower seeds and flowers, few phytochemical studies on the safflower leaf, which is used as wild edible green, are avaliable. As a part of a continuing search for natural antioxidants, we previously found that the methanolic extract of the safflower leaf has strong antioxidative activity, and this activity is increased in relation to the roasting and steamming process (Park *et al.*, 2001). This prompted us to investigate the major antioxidative constituents of the safflower leaf. This paper reports the isolation and identification of flavonoids from the safflower leaf, and further describes their antioxidative activity against 2-deoxyribose degradation and microsomal lipid peroxidation induced by hydroxyl radical formed via a Fenton-type reaction.

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MATERIALS AND METHOD

Instrumental and general techniques

UV and IR spectra were obtained on a photodiode array UV-vis spectrophotometer (1100 Sinco, Korea) and a FT-IR spectrometer (IFS 120 HR, Bruker, Germany), respectively. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured in DMSO-d_6 on a Unity Plus 500 spectrometer (Varian, USA) and chemical shifts are given as δ values with tetramethylsilane (TMS) as an internal standard. Fast-atom bombardment mass spectrometry (FAB-MS) was recorded on a JMS-700 mass spectrometer (ion source, Xe atom beam; accelerating voltage, 10 kV, JEOL, Japan), using glycerol and *m*-butylalcohol as a mounting matrix. Column chromatography was performed on silica gel (70-230 mesh, Merck, Damstadt, Germany) and Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Damstadt, Germany) and preparative silica C18 (0.25 mm, Merck, Damstadt, Germany) plates, and spots visualized under UV light at 254 and 366 nm and or sprayed with 20% H_2SO_4 solution, followed by heating. All solvents used for this study were of analytical and HPLC grades.

Chemicals

Thiobarbituric acid (TBA), 2-deoxyribose, H_2O_2, bovine serum albumin (BSA) and $FeSO_4$ were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), ethylene- diaminetetraacetic acid (EDTA) and α-tocopherol were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Plant materials

The leaves of safflower (*Carthamus tinctorius* L.) were directly harvested early May at an herb garden in Uisong Medicinal Plant Experiment Station, Gyeongbuk, Korea. A voucher specimen has been retained in the Herbarium of Uisong Medicinal Plant Experiment Station.

Extraction and isolation

The air-dried safflower leaves (120 g) were roasted at 150°C for 2 min and then extracted with boiling water (2.5 l × 2) under reflux at 90°C for 2 h. The filtrate was fraction ated with BuOH (3l × 2) and evaporated to dryness under reduced pressure. The BuOH soluble fraction (6.5 g) was chromatographed on silica gel column with $CHCl_3$-MeOH-H_2O (65:35:5, v/v) as an eluent to provide two-hundred fractions of 5 ml, and then combined together into seven groups according to TLC separations [eluent: $CHCl_3$-MeOH (2:1, v/v)]. Among the seven fractions (F1-F7), Fraction **2** (1.1 g) was repeatedly chromatographed on Sephadex LH-20 column chromatography with MeOH to obtain compound **1** (254 mg, 0.21%) and compound **2** (136 mg, 0.11%). The F3-F5 fractions (1.4 g) were combined and submitted to Sephadex LH-20 chromatography with 90% aqueous MeOH, Five main subfractions (A-E) were obtained according to SiO_2 TLC separation with $CHCl_3$-MeOH (2:1, v/v). Subfraction B and C were further subjected on C18 PTLC separation with MeOH-H_2O-HOAc (60:39:1, v/v) to yield compound **3** (267 mg, 0.22%, R*f*=0.53) and compound **4** (154 mg, 0.13%, R*f*=0.24). Subfractions D and E were also combined and subjected to the same purification procedure on C18 PTLC chromatography to afford compound **5** (134 mg, 0.11%, R*f*=0.64) and compound **6** (312 mg, 0.26%, R*f*=0.40). Finally, fractions 6 and 7 (0.51 g) were combined and subjected to Sephadex LH-20 column chromatography with 90% aqueous MeOH to obtain compound **7** (19 mg, 15.8 mg%) and compound **8** (50 mg, 41.7 mg%). Herein, the detailed spectral and chemical data of three compounds **4**, **6** & **7**, excluding the five well-known flavonoids (**1**-**3**, **5** & **8**) were as follows;

Compound **4** [luteolin 7-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside]: yellow needles, mp. 142~149°C; IR ν_{max} (cm^{-1}); 3424 (OH), 1730 (aliphatic ketone), 1653 (C=O), 1617 and 830 (phenyl), 1027 (=C-O-); UV (MeOH) λ_{max}: 246, 258 (sh), 350 nm, UV (NaOMe) λ_{max}: 253, 360 (sh), 390, 407 (sh) nm, UV (NaOAc) λ_{max}: 247, 260 (sh), 350 nm, UV (NaOAc+H_3BO_3) λ_{max}: 248, 369 nm, UV ($AlCl_3$) λ_{max}: 263, 290 (sh), 395 (sh), 424 nm, UV ($AlCl_3$+HCl) λ_{max}: 262, 290 (sh), 360 (sh), 391 nm; FAM-MS m/z 491 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm) δ 7.45 (dd, *J*=2.0, 8.5, H-6'), 7.42 (d, *J*=2.0, H-2'), 6.91 (d, *J*=8.5, H-3'), 6.77 (d, *J*=2.0, H-8), 6.76 (s, H-3), 6.44 (d, *J*=2.0, H-6), 5.12 (d, *J*=7.0, H-1"), 4.34 (dd, *J*=2.0, 11.5, H-6_a"), 4.06 (br.d, *J*=11.5, H-6_b"), 3.74 (t, *J*=8.5, H-3"), 3.34 (brs, H-5"), 3.31 (t, *J*=9.3, H-2"), 3.17 (s, H-4"), 2.01 (Ac-H); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ182.14 (C-4), 170.44 (acetyl C=O), 164.69 (C-2), 162.89 (C-7), 161.34 (C-5), 157.14 (C-9), 150.15 (C-4'), 146.01 (C-3'), 121.57 (C-1'), 119.35 (C-6'), 116.18 (C-5'), 113.76 (C-2'), 105.63 (C-10), 103.38 (C-3), 99.82 (C-1"), 99.80 (C-6), 94.93 (C-8), 76.39 (C-3"), 74.09 (C-5"), 73.24 (C-2"), 70.05 (C-4"), 63.60 (C-6"), 20.79 (CH_3).

Compound **6** [quercetin 7-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside]: yellow needles, mp. 149~154°C; IR ν_{max} (cm^{-1}); 3422 (OH), 1722 (aliphatic ketone), 1653 (C=O), 1597 and 830 (phenyl), 1071 (=C-O-); UV (MeOH) λ_{max}: 245, 262 (sh), 370 nm, UV (NaOMe) λ_{max}: 258, 427 nm, UV (NaOAc) λ_{max}: 247, 260 (sh), 371, 410 (sh) nm, UV (NaOAc+H_3BO_3) λ_{max}: 250, 390, 410 (sh) nm, UV ($AlCl_3$) λ_{max}: 248 (sh), 262, 458 nm, UV ($AlCl_3$+HCl) λ_{max}: 260, 360 (sh), 429 nm; FAM-MS m/z 507 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm) δ 7.73 (d, *J*=2.0, H-2'), 7.56

(dd, J=2.0, 10.0, H-6'), 6.90 (d, J=10.0, H-3'), 6.75 (d, J=2.0, H-8), 6.42 (d, J=2.0, H-6), 5.11 (d, J=8.0, H-1"), 4.36 (dd, J=2.0, 11.5, H-6_a"), 4.06 (br.d, J=11.5, H-6_b"), 3.75 (t, J=9.8, H-3"), 3.34 (brs, H-5"), 3.30 (t, J=9.5, H-2"), 3.18 (s, H-4"), 2.01 (Ac-H); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ 175.92 (C-4), 170.11 (acetyl C=O), 162.32 (C-7), 160.24 (C-5), 155.59 (C-9), 147.79 (C-2), 147.48 (C-4'), 144.96 (C-3'), 136.01 (C-3),121.70 (C-1'), 119.88 (C-6'), 115.42 (C-2'), 115.25 (C-5'), 104.62 (C-10), 99.53 (C-1"), 98.65 (C-6), 94.14 (C-8), 76.07 (C-5"), 73.77 (C-3"), 72.92 (C-2"), 69.72 (C-4"), 63.31 (C-6"), 20.49 (CH_3).

Compound **7** (acacetin 7-*O*-β-D-glucuronide): pale yellow needles, mp. 268~272°C; IR ν_{max} (cm^{-1}); 3435 (OH , 1720 (C=O), 1621 (C=O), 1489 and 828 (phenyl), 1440 (Ar-OMe), 1276 (C-O), 1029 (=C-O-); UV (MeOH) λ_{max}: 260, 322 nm, UV (NaOMe) λ_{max}: 274, 380 (sh) nm, UV (NaOAc) λ_{max}: 260, 317 nm, UV (NaOAc+H_3BO_3) λ_{max}: 260, 316 nm, UV ($AlCl_3$) λ_{max}: 265, 290 (sh), 337 nm, UV ($AlCl_3$+HCl) λ_{max}: 265, 290 (sh), 338, 380 (sh) nm; FAM-MS m/z 461 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz, ppm) δ 8.07 (d, J=9.0, H-2' & H-6'), 7.13 (d, J=9.0, H-3' & H-5'), 6.95 (s, H-3), 6.86 (d, J=1.5, H-8), 6.45 (d, J=1.5, H-6), 5.07 (d, J=7.5, H-1"), 3.59 (d, J=9.5, H-5"), 3.30 (t, J=8.3, H-3" , 3.24 (t, J=8.8, H-2"), 3.18 (t, J=9.3, H-4"); ^{13}C-NMR (DMSO-d_6, 125 MHz, ppm) δ 182.24 (C-4), 172.34 (C-6"), 163.98 (C-2), 163.40 (C-7), 162.66 (C-4'), 161.26 (C-5), 157.20 (C-9), 128.65 (C-2' & C-6), 122.89 (C-1'), 114.85 (C-3' & C-5), 105.55 (C-10), 103.99 (C-3), 99.96, 99.89 (C-6', 95.01 (C-8), (C-1"), 76.72 (C-3"), 73.87 (C-5"), 73.14 (C-2"), 72.13 (C-4"), 55.80 (OCH_3).

HPLC analysis

HPLC was performed on a Gilson 506B HPLC System coupled with Gilson 170 UV-vis detector, and Gilson 231 XL autosampler with a 10 μl loop. HPLC analysis was carried out using a Pro C18 column (5 μm, 46 × 250 mm, YMC Inc., USA) with Guard-Pak HPLC *precolumn inserts*. The separation was conducted using a linear gradient from 0.03% v/v H_3PO_4 in 20% MeOH to 80% MeOH for 40 min at a flow rate of 1.0 ml/min with detection at UV_{350} nm. Peaks were identified by co-chromatography with authentic samples isolated previously. Retention times (min): Comp. **1** (30.69); Comp. **2** (29.05); Comp. **3** (17.60); Comp. **4** (28.42); Comp. **5** (15.55); Comp. **6** (27.08); Comp. **7** (33.75) and Comp. **8** (11.69).

2-Deoxyribose degradation assay

The procedure of Halliwell *et al.* (1987) was followed with a slight modification. The following reagents were added to the glass tube in the order shown: 0.1 mM Fe^{+2}/EDTA (0.2 ml), 10 mM 2-deoxyribose (0.2 ml), test solution (0.02 ml, various concentrations), 0.1 M potassium phosphate buffer (1.38 ml, pH 7.4), 30% H_2O_2 (0.2 ml). The reaction mixtures were incubated at 37°C for 4 h in a shaking water bath, and peroxidation was determined by using the thiobarbituric acid (TBA) method described by Ohkawa *et al.* (1979). All experiments were performed in triplicate. A curve plotting concentration against percentage inhibition was used to calculate half the maximal inhibition concentration (IC_{50}).

Lipid peroxidation assay

Lipid peroxidation was induced in rat liver microsomes by H_2O_2-$FeSO_4$ according to the method of Yokozawa *et al.*. (1999), with minor modifications. Microsomes were prepared from livers of male Sprague-Dawley rats weighing 250-300 g by differential centrifugation (Slater and Sawyer, 1971). Microsomal pellets were suspended in a 50 mM phosphate buffer (pH 7.4). Aliquots of this microsomal suspension were stored at -70°C and thawed before use. Protein was determined by the method of Lowry *et al.* (1951) using BSA as a standard. The final protein concentration of the microsomal suspensions was adjusted to 10 mg protein/ml. A mixture of microsomal suspension (0.5 ml, 2 mg protein/ml), 0.5% H_2O_2 (0.3 ml) and 10 mM $FeSO_4$ (0.3 ml) solution and sample (0.1 ml, final concentration 0.1~10 μM) was incubated at 37°C for 30 min, and then peroxidation was measured by the TBA method as described above. All results were performed in three replicates. Statistical analysis was performed using Duncan's multiple range test (SAS, 1985).

RESULTS AND DISCUSSION

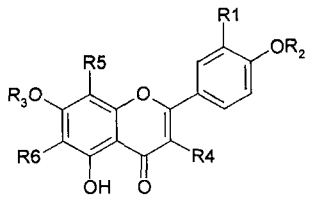
The *n*-butanol soluble material of the boiling extract was subjected to repeated chromatographic separations on silica gel, Sephadex LH-20 and reverse-phase silica gel to yield eight flavonoids, **1-8** in the pure state. The structures of the known flavonoids **1-3**, **5** and **8** were determined by comparison of their UV, IR, MS and NMR spectral data with those reported in the literature; Compounds **1** and **2** were identified as luteolin (Kim *et al.*, 1998) and quercetin (Kim *et al.*, 1995), respectively. Compounds **3** and **5** were elucidated as luteolin-7-*O*-β-D-glucopyranoside and quercetin 7-*O*-β-D-glucopyranoside (Williams and Harborne, 1994), respectively, and compound **8** as apigenin-6,8-di-*C*-glucopyranoside (Hilsenbeck *et al.*, 1983; Iwashina *et al.*, 1990). Meanwhile, Comp. **4** and Comp. **7** were characterized as luteolin-7-β-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside and acacetin-7-*O*-β-D-glucuronide, respectively, which have only been reported only in some plants (Okigawa *et al.*, 1970; Mizuno *et al.*, 1987; Williams and Harborne, 1994; Williams *et al.*, 2001). Herein, we identified the structures of Comp. **4**, **6**, and **7**, which have not been reported on precise assignments for FAB-MS

and 1D- & 2D-NMR spectral data.

Compound **4** yielded a protonated molecule $[M+H]^+$ at *m/z* 491.12 in the positive-ion high-resolution (HR) FAB mass spectrum consistent with the molecular formula $C_{23}H_{23}O_{12}$ (calcd. *m/z* 491.1190). Two significant fragment ion peaks were observed at *m/z* 448 [491 (M+H)-43 $(CH_3CO)]^+$ and 286 [448-162 (hexose)]$^+$, indicating the presence of agylcone luteolin and acetylglucoside. The ^{1}H- and ^{13}C-NMR spectra of compound **4** were similar to those of compound **3**, except one acetic residue was attached to a glucose moiety of **4** (33). Compared to compound **3**, the H-6" (δ 4.34, 4.06) and C-6" (δ 63.60) signals of the glucosyl unit of compound **4** were shifted downfield, suggesting a substitution of acetic residue at the C-6" position of glycosyl moiety. This conclusion was also supported by HMQC and HMBC spectra, in which significant correlations were observed between the CH_3 proton signal (δ 2.01) of the acetyl group and its carbon signal (δ 20.79), H-1" (δ 5.12) and C-7 (δ 162.89), and H-6" (δ 4.34 and 4.06) and the carbonyl carbon (δ 170.44). On the basis of these results, compound **4** was identified as luteolin-7-β-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside (Mizuno *et al.*, 1987).

Compound **6** provided a $[M+H]^+$ at *m/z* 507.04 in the positive HRFAB-MS spectrum, corresponding to the molecular formula $C_{23}H_{23}O_{13}$ (calcd. *m/z* 507.1145). Two major fragment ion peaks at *m/z* 464 [507 (M+H)-43 $(CH_3CO)]^+$ and 302 [464-162 (hexose)]$^+$ indicated the presence of agylcone quercetin and acetylglucoside. The ^{1}H- and ^{13}C-NMR spectrum of compound **6** were closely analogous to those of compound **5**, except for the presence of significant signals of an acetyl group. A comparison of the spectral data of compound **5** with that of Compound **6** showed that the H-6" (δ 4.36, 4.06) and C-6" (δ 63.31) signals of glucosyl moiety were shifted downfield, suggesting that the acetic unit was attached to the C-6" of glycosyl moiety. This fact was secured by HMBC cross peak between H-6" (δ 4.36 and 4.06) and the acetyl ketone carbon (δ 170.11). Additionally, the presence of one acetic residue of Compound **6** was confirmed by the HMQC spectrum, in which one CH_3 proton signal (δ 2.01) was correlated with its carbon signal (δ 20.49). Finally, the HMBC spectrum showed a correlation between the proton at δ 5.12 (H-1") and C-7 (δ 162.89), indicating glucose should be attached to C-7 of luteolin skeleton. From these results, Compound **6** was identified as quercetin-7-*O*-(6"-*O*-acetyl)-β-D-glucopyranoside, a new na-tural flavonol.

Compound **7** gave a $[M+H]^+$ at *m/z* 461 in the positive FAB-MS spectrum, together with major fragment ions at *m/z* 285 [(M+H)-176]$^+$, indicative of acacetin nucleus with glucuronic acid moiety (Flamini *et al.*, 2001). The ^{1}H- and ^{13}C-NMR of **7** were quite consistent with those of acacetin



	R_1	R_2	R_3	R_4	R_5	R_6
Compound 1 :	OH	H	H	H		
Compound 2 :	OH	H	H	OH		
Compound 3 :	OH	H	glucose	H		
Compound 4 :	OH	H	glucose	OH		
Compound 5 :	OH	H	acetylglucose	H		
Compound 6:	OH	H	acetylglucose	OH		
Compound 7:	H	CH_3	glucuronide	H		
Compound 8:	H	H	H	H	glucose	glucose

Fig. 1. *Chemical structures of compounds* **1-8**

glucoside (Okigawa *et al.*, 1970). As compared to acacetin 7-glucoside, compound **7** indicated a lower field shift of H-5" (δ 3.59) of the glucosyl unit together with a C-6" signal at 172.34, suggesting the substitution of a carboxylic acid at C-6" position of glycosyl moiety (Markham *et al.*, 1978; Markham and Geiger, 1994). This fact was reinforced by the HMBC cross peak between H-4" (δ 3.18) or H-5" (δ 3.59) and C-6" (δ 172.34). In addition, the HMQC spectrum was allowed to characterize the presence of one methoxyl residue, in which one OCH_3 proton signal (δ 3.87) was correlated with its carbon signal (δ 55.8). Finally, the HMBC spectrum showed a correlation between H-1" (δ 5.07) and C-7 (δ 163.40), indicating glucuronic acid should be attached to C-7 of acacetin skeleton. Thus, the structure of compound **7** was elucidated as acacetin-7-*O*-β-D-glucuronide. This is the first report of the isolation and identification of eight compounds (**1-8**) from safflower leaf. The chemical structures of the eight compounds are given Fig. 1.

The hydroxyl radical of several active oxygen radicals has a high and indiscriminate activity and can slowly cause severe damage to susceptible biomolecules. However, this damage can be modulated by dietary antioxidant flavonoids. Until now, despite the fact that numerous studies of hydroxyl radical (· OH) scavenging activity of flavonoids have been performed (Husain *et al.*, 1987; Puppo, 1991; Salah *et al.*, 1995), information on the · OH scavenging activity of acetyl flavonoids remains very limited. Eight flavonoids including acetyl flavonoids isolated from safflower leaf were tested for their antioxidant properties using hydroxyl radical-induced 2-deoxyribose degradation and lipid peroxidation of rat liver microsomes, and the results are shown in Table 1. All flavonoids acted as a hydroxyl radical scavenger, and significantly inhibited 2-deoxyribose degradation and lipid peroxidation in a dose-dependent manner. Among these flavonoids, quercetin-acetyl-glucoside (**6**) and luteolin-

Table 1. Inhibitory activity (IC_{50}) of compounds **1-8** from safflower leaf against 2-deoxyribose degradation and rat liver microsomal lipid peroxidation induced by hydroxyl radical generated via a Fenton-type reaction

Compound	IC_{50} (μM)	
	2-Deoxyribose	Lipid peroxidation
Compound **1**	3.02[c]	1.23[c]
Compound **2**	3.77[b]	1.11[c]
Compound **3**	2.98[c]	2.87[b]
Compound **4**	2.91[c]	0.95[c]
Compound **5**	3.81[b]	2.66[b]
Compound **6**	3.73[b]	0.85[c]
Compound **7**	8.42[a]	5.64[a]
Compound **8**	8.67[a]	6.43[a]
α-Tocopherol		0.98[c]

IC_{50} values represent mean of three independent determinations. Standard deviations were omitted for simplicity. Values with the different superscript letter in each column are significantly different at $P < 0.05$. α-Tocopherol was used as a positive reference.

acetyl-glucoside (**4**) showed the most potent antioxidative activities in two *in vitro* antioxidative model systems; in particular, their activities were comparable to that of α-tocopherol, a reference antioxidant against the lipid peroxidation in rat liver microsomes. In addition, luteolin (**1**), quercetin (**2**) and their corresponding glycosides (**3-6**) showed a strong hydroxy radical scavenging activity without big differences in scavenging efficiencies among them. However, acacetin glucuronide (**7**) and apigenin glycoside (**8**) had a weaker activity than those of the six other flavonoids. Thus, it is interesting to note that the presence of the 6"-acetyl group increases the hydroxy radical scavenging activity in the lipid membrane model, although the participation of the 6"-acetyl group in the antiperoxidative and superoxide scavenging activities of flavonoids has already been established in previous work (Rice *et al.*, 1992; Izumi *et al.*,1997; Lee *et al.*, 1999). Moreover, it was found that the glycosylation of the hydroxyl group in flavonoids decreased antioxidative activity due to a low affinity for biological lipid membranes (Saija et al., 1995). From this data, a comparison of hydroxyl radical scavenging activity between flavones and flavonols demonstrated the importance of the presence of the acetyl group and the *ortho*-catechol moiety in ring B, irrespective of the addition of a hydroxyl group at the C-3 position. Thus, these results suggest that the safflower leaf containing peculiar acetyl flavonoid glycosides, together with well-known antioxidative flavonoids, luteolin and quercetin (Cheng and Breen, 2000), may be useful as a potential source of natural antioxidant capable of inhibiting a variety of pathological diseases induced by peroxidation of membrane lipids. Further study is also required to investigate the mechanism of antioxidative activity of eight flavonoids *in vivo*.

ACKNOWLEDGEMENTS

This research was partly supported by a 2001 grant from the Catholic University of Daegu. We are grateful to Dr. Kim Sung Hong and Ms. Chae Seen Ae, Korea Basic Science Institute, Daegu, Korea, for NMR and FABMS measurements.

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