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Antioxidant Constituents from the Leaves of *Cedrela sinensis* A. Juss

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ABSTRACT : Phytochemical study on the EtOAc fraction from the MeOH extract of the leaves of *Cedrela sinensis* led to the isolation of five known phenolic compounds (1-5), whose structures were identified as (+)-catechin (1), kaempferol-3-*O*-α-L-rhamnopyranoside (2), quercetin (3), quercetin-3-*O*-α-L-rhamnopyranoside (4), and quercetin-3-*O*-β-D-glucopyranoside (5), respectively, by comparing their spectral (UV, IR, ^{1}H and ^{13}C-NMR, and ESI-MS) and physicochemical data with those reported in the literature. Among the isolated compounds (1-5), compounds 1 and 3-5 exhibited significant DPPH radical scavenging effects with IC_{50} values ranging from 21.3 ± 1.4 to 38.1 ± 3.2 μM as well as superoxide anion radical scavenging effects with IC_{50} values ranging from 9.4 ± 0.7 to 21.2 ± 3.6 μM. Furthermore, compounds 1 and 3-5 also exhibited considerable inhibitory effects on LDL peroxidation induced by either Cu^{2+} or AAPH with IC_{50} values ranging from 1.4 ± 0.4 to 11.9 ± 1.4 μM. These results indicated that flavonoids are the major constituents of *C. sinensis* and considered to be antioxidant principles of this plant.

Key words **:** *Cedrela sinensis*, Flavonoids, Antioxidant, DPPH, Superoxide, LDL oxidation

INTRODUCTION

In recent years, there has been increasing interest in antioxidants derived from natural products. Numerous epidemiological and experimental studies have revealed a clear relationship between antioxidants and diseases such as cancer, atherosclerosis, and cardiovascular disease (Greenwald & McDonald, 1997; Lindquist *et al.*, 2000). Moreover, antioxidants are found in many natural products, which include natural phenolics and vitamins, are reported to be more safe and powerful than synthetic antioxidants such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) (Wanasundara & Shahidi, 1998; Tang *et al.*, 2001). For these reasons, demand for natural antioxidants has been increased.

Cedrela sinensis A. Juss (Meliacea) is a medicinal plant, which is widely distributed in China and cultivated in Korea. The fresh young leaves and shoots have been used as an effective nutritious food in Korea for a long time. All most every part of *C. sinensis*, including seeds, root bark, peptioles, and leaves, has a medicinal effect (Edmonds & Staniforth, 1998). In fact, the leaves and stems of this plant have been used for the treatment of itch, dysentery, and enteritis in oriental medicine (Lee, 1985). In addition, the powdered root has been used as a corrective, and the fruits have been used as an astringent and for the treatment of eye infection (Stuart, 1911; Perry, 1980). Recently, it was reported that the crude extract of *C. sinensis* can induce apoptosis of cancer cells (Chang *et al.*, 2002), enhance lipolysis of differentiated 3T3-L1 adipocyte and its uptake of glucose (Hsu *et al.*, 2003; Yang *et al.*, 2003), reduce plasma glucose in diabetic rats (Yu, 2002), and inhibit Leydig cell steroidogenesis (Poon *et al.*, 2005). As a part of our continuing study on the bioactive principles of this plant, we have found that the EtOAc-soluble fraction of *C. sinensis* exhibited a considerable antioxidant effect on DPPH assay. This prompted us to investigate the major antioxidant constituents of this plant, and resulted in the isolation of five phenolic compounds. The present paper reports the isolation and structural identification of these compounds and their free radical scavenging effects on DPPH and superoxide anion radicals, as well as their inhibitory effects on low-density lipoprotein (LDL) peroxidation induced by Cu^{2+} or AAPH using the TBA method.

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MATERIALS AND METHODS

Plant materials

The leaves of *C. sinensis* were collected in Daejeon, Korea, in October 2005, and identified by Prof. KiHwan Bae. A voucher specimen (CNU 1269) has been deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Instruments and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), xanthine, xanthine oxidase, nitro blue tetrazolium (NBT), 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), thiobarbituric acid (TBA), α-tocopherol (vitamin E), and butylated hydroxyl toluene (BHT) were purchased from Sigma Co., U. S. A. Etylenediaminetetraacetatic acid (EDTA) and tricloroacetic acid (TCA) were obtained from Daejung Chemical, Ltd., Korea.

Melting points were determined on an Electrothemal apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO 100 IR spectrophotometer. UV spectra were obtained a JASCO V-550 UV/VIS spectrometer. ESI-MS were registered using a HP 1100 series LC/MSD spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker DRX-300 spectrometer and chemical shift are expressed as δ values using TMS unit as an internal standard. Column chromatography was performed by using silica gel (Kieselgel 70-230 mesh and 230-400 mesh, Merck), Sephadex LH-20 (Amersham Biosciences), and Lichroprep RP-18 (Merk). Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} (0.25 ㎜, Merck) and RP-18 F_{254s} plates (0.25 ㎜, Merck). Spots were detected by UV light (254 ㎚) and spraying 10% H_2SO_4 followed by heating. Analytical and quantitative HPLC were used performed on a Shimadzu SCL-10A system controller with LC-10AD pump; SPD-10A UV-VIS multiwavelength detector; ZORBAX eclipse XID-C_{18} (2.1 ㎜ × 150 ㎜. 5 μm) column. Preparative MPLC was carry out on YAMZEN MPLC system with pump 540; detector Prep UV-10V; ULTRA PACK ODS-S-50A (11 ㎜ × 300 ㎜).

Extraction and isolation

The dried leaves of *C. sinensis* (6.0 ㎏) were extracted three times with MeOH at room temperature for 7 days, filtered and concentrated to afford a MeOH extract (526.0 g). The MeOH extract was suspended in H_2O and then partitioned successively with hexane, EtOAc, and BuOH to afford a hexane-soluble fraction (170 g), an EtOAc-soluble fraction (90 g), and a BuOH-soluble fraction (150 g), respectively. As a result of measuring the antioxidant effect of the *C. sinensis* extract and its solvent-fractions, the EtOAc-soluble fraction exhibited the strongest antioxidant effect with an IC_{50} value of 22.0 ± 1.5 ㎍/㎖ on DPPH assay. Accordingly, the EtOAc-soluble fraction (90 g) was subjected to silica gel column chromatography (100 × 8.5 ㎝) eluting with a gradient of CHC_3-MeOH (100 : 1 to 1 : 1) to afford four fractions (Fr. 1~4). Fr. 2 was chromatographed on a silica gel column (50 × 4.5 ㎝) using CHC_3–MeOH (20 : 1 to 5 : 1) to afford seven subfrations (Fr. 2-1~2-7). Fr. 2-6 and Fr. 2-7 were rechromatographed on Sephadex LH-20 (30×3 ㎝) using MeOH–H_2O (7 : 3) and MeOH–H_2O (6 : 4) to yield compounds **1** (20 ㎎) and **2** (100 ㎎), respectively. Fr. 4 was further chromatographed on a silica gel column (50 × 4.5 ㎝) using $CHCl_3$-MeOH (20 : 1 to 2 : 1) to afford three subfrations (Fr. 4-1~4-3). Fr. 4-1 was applied to preparative MPLC (ODS-S-50A column, 11 × 300 ㎜, Yamazen corporation; UV, 254 ㎚; flow rate, 4 ㎖/min) using MeOH-H_2O (6 : 4) to yield compound **3** (220 ㎎). Compound **4** (350 ㎎) was obtained from Fr. 4-2 after chromatographed on Sephadex LH-20 (3×30 ㎝) using MeOH–H_2O (1:1). Fr. 4-3 was further chromatographed on an RP-18 column (2.5×30 ㎝) using MeOH–H_2O (1 : 1) to yield compound **5** (540 ㎎).

Compound 1 – White amorphous powder; mp: 175-176 ℃; $[\alpha]_D$ +16.0° (*c* 0.1, MeOH); UV λ_{max} ㎚ (MeOH): 228, 283; IR (KBr) ν_{max} ㎝$^{-1}$: 3300 (OH), 1610, 1520 (aromatic C = C), 1280, 1140; ESI-MS *m/z*: 313 $[M+Na]^+$; ^{1}H-NMR (300 MHz, CD_3OD): 6.83 (1H, d, *J* = 1.8 Hz, H-2'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.72 (1H, dd, *J* = 1.8, 8.0 Hz, H-6'), 5.92 (1H, d, *J* = 2.0 Hz, H-6), 5.85 (1H, d, *J* = 2.0 Hz, H-8), 4.56 (1H, d, *J* = 7.5 Hz, H-2), 3.97 (1H, ddd, *J* = 5.4, 7.5, 8.0 Hz, H-3), 2.85 (1H, dd, *J* = 5.4, 16.0 Hz, H-4β), 2.50 (1H, dd, *J* = 8.0, 16.0 Hz, H-4α); ^{13}C-NMR (75 MHz, CD_3OD): 82.8 (C-2), 68.8 (C-3), 28.5 (C-4), 157.6 (C-5), 96.2 (C-6), 157.8 (C-7), 95.5 (C-8), 156.9 (C-9), 100.8 (C-10), 132.2 (C-1'), 115.2 (C-2'), 146.2 (C-3'), 146.2 (C-4'), 116.1 (C-5'), 120.0 (C-6').

Compound 2 – Yellow amorphous powder; mp: 172-175 ℃; $[\alpha]_D$ –184.0° (*c* 0.1, MeOH); UV λ_{max} ㎚ (MeOH): 269, 347; IR (KBr) ν_{max} ㎝$^{-1}$: 3350 (OH), 1650 (C = O), 1610, 1510, 1450 (aromatic C = C), 1365, 1170 (glycosidic C-O); ESI-MS *m/z*: 455 $[M+Na]^+$; ^{1}H-NMR (300 MHz, CD_3OD): 7.73 (2H, d, *J* = 8.0 Hz, H-2', 6'), 6.91 (2H, d, *J* = 8.0 Hz, H-3', 5'), 6.32 (1H, d, *J* = 1.2 Hz, H-8), 6.15 (1H, d, *J* = 1.2 Hz, H-6), 5.36 (1H, d, *J* = 1.6 Hz, rham-1), 0.92 (3H, d, *J* = 5.5 Hz, rham-6); ^{13}C-NMR (75 MHz, CD_3OD): 159.1 (C-2), 136.1 (C-3), 179.4 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10), 122.5 (C-1'), 131.9 (C-2', 6'), 116.4 (C-3', 5'), 161.4 (C-4'), 103.4 (rham-1), 71.9 (rham-2), 72.0 (rham-3), 73.1 (rham-4), 71.8 (rham-5), 17.6 (rham-6).

Compound 3 – Yellow amorphous powder; mp: 313-314 ℃; UV λ_{max} nm (MeOH): 267, 371; IR (KBr) ν_{max} cm^{-1}: 3380 (OH), 1676 (C = O), 1608, 1516, 1455 (aromatic C = C); ESI-MS *m/z*: 325 $[M+Na]^+$; ^{1}H-NMR (DMSO-d_6, 300 MHz): 7.67 (1H, d, *J* = 2.1 Hz, H-2), 7.54 (1H, dd, *J* = 2.1, 8.4 Hz, H-6), 6.88 (1H, d, *J* = 8.4 Hz, H-5), 6.40 (1H, d, *J* = 1.8 Hz, H-8), 6.18 (1H, d, *J* = 1.8 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 75 MHz): 147.6 (C-2), 136.6 (C-3), 176.7 (C-4) 161.6 (C-5), 99.0 (C-6), 164.8 (C-7), 94.2 (C-8), 157.0 (C-9), 103.8 (C-10), 122.8 (C-1'), 116.4 (C-2'), 145.9 (C-3'), 148.6 (C-4'), 115.9 (C-5'), 120.8 (C-6').

Compound 4 – Yellow amorphous powder; mp: 179-181 ℃; $[\alpha]_D$ –178.0° (*c* 0.1, MeOH); UV λ_{max} nm (MeOH): 255, 353; IR (KBr) ν_{max} cm^{-1}: 3320 (OH), 1660 (C = O), 1610, 1500, 1450 (aromatic C = C), 1360, 1140 (glycosidic C-O); ESI-MS *m/z*: 471 $[M+Na]^+$; ^{1}H-NMR (300 MHz, CD_3OD): 7.33 (1H, d, *J* = 2.0 Hz, H-2'), 7.30 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 6.90 (1H, d, *J* = 8.0 Hz, H-5'), 6.34 (1H, d, *J* = 2.1 Hz, H-8), 6.20 (1H, d, *J* = 2.1 Hz, H-6), 5.34 (1H, d, *J* = 1.5 Hz, rham-1), 4.22 (1H, dd, *J* = 1.7, 3.0 Hz, rham-2), 3.44-3.30 (3H, m, rham-3, 4, 5), 0.94 (3H, d, *J* = 6.0 Hz, rham-6); ^{13}C-NMR (75 MHz, CD_3OD): 159.2 (C-2), 136.2 (C-3), 179.5 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10), 122.8 (C-1'), 116.9 (C-2'), 146.3 (C-3'), 149.7 (C-4'), 116.3 (C-5'), 122.9 (C-6'), 103.5 (rham-1), 72.1 (rham-2), 72.0 (rham-3), 73.2 (rham-4), 71.8 (rham-5), 17.6 (rham-6).

Compound 5 – Yellow amorphous powder; mp: 232-234 ℃; $[\alpha]_D$ –12.5° (*c* 0.1, MeOH); UV λ_{max} nm (MeOH): 257, 355; IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 1660 (C = O), 1610, 1500, 1450 (aromatic C = C), 1360, 1140 (glycosidic C-O); ESI-MS *m/z*: 487 $[M+Na]^+$; ^{1}H-NMR (300 MHz, CD_3OD): 7.71 (1H, d, *J* = 2.0 Hz, H-2'), 7.56 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 6.86 (1H, d, *J* = 8.0 Hz, H-5'), 6.34 (1H, d, *J* = 2.1 Hz, H-8), 6.16 (1H, d, *J* = 2.1 Hz, H-6), 5.23 (1H, d, *J* = 7.3 Hz, glc-1), 3.72 (1H, dd, *J* = 12.0, 2.1 Hz, glc-6a), 3.58 (1H, dd, *J* = 12.0, 5.1 Hz, glc-6b), 3.51-3.22 (4H, m, glc-2, 3, 4, 5); ^{13}C-NMR (75 MHz, CD_3OD): 158.4 (C-2), 135.6 (C-3), 179.4 (C-4), 162.9 (C-5), 99.8 (C-6), 165.9 (C-7), 94.7 (C-8), 158.9 (C-9), 105.6 (C-10), 123.0 (C-1'), 117.5 (C-2'), 145.8 (C-3'), 149.7 (C-4'), 115.9 (C-5'), 123.1 (C-6'), 104.3 (glc-1), 75.7 (glc-2), 78.1 (glc-3), 71.1 (glc-4), 78.0 (glc-5), 62.5 (glc-6).

DPPH radical scavenging assay

The DPPH assay was performed as described previously (Taco *et al.*, 1994). Briefly, 5 ㎕ of each sample, dissolved in DMSO, was added to 195 ㎕ of 150 μM DPPH in ethanol in 96 well plates. The solution was mixed for 1 min and incubated at room temperature for 30 min. The absorbance of reaction mixture was measured at 517 nm on a microplate reader. The free radical scavenging activity was expressed as follow:

DPPH radical scavenging activity (%) = [(Ac-As) / (Ac-Ab)]×100

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (DMSO). The IC_{50} value was defined as the concentration of sample required to scavenge 50% of DPPH radicals.

Superoxide anion radical scavenging assay

The superoxide assay was performed as described previously (Beauchamp & Fridovich, 1971). The 495 ㎕ assay mixture consisted of 50 mM sodium carbonate buffer (pH 7.8), containing 0.1 mM xanthine and 25 μM nitro blue tetrazolium (NBT). The reaction was initiated by addition of 5 ㎕ 20 nM xanthine oxidase in the presence or absence of each compound. The increase in absorbance at 560 nm was read after 5 min on a spectrophotometer (Shimadzu UV-1240, Tokyo, Japan). Superoxide radical scavenging activity was expressed as follow:

Superoxide anion radical scavenging activity (%) = [(Ac-As) / (Ac-Ab)]×100

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (where the xanthine oxidase was not added). The IC_{50} value was defined as the concentration of sample required to decrease 50% of NBT reduction.

Preparation of low density lipoprotein (LDL)

Plasma was obtained from fasted healthy normalipidemic individual. LDL was isolated as described previously (Nicole & Mavis, 1998). Plasma was centrifuged at 43800 rpm for 20 hrs at 4 ℃ in Beckman T8-M ultracentrifuge and then chylomicron and very low-density lipoprotein floated to the top of tube was removed. Other infranatants was collected, adjusted to d = 1.063 g/㎖ using NaBr and centrifuged at 43800 rpm for 28 hrs at 4 ℃. The top layer of LDL was collected and dialyzed against 10 mM phosphate saline buffer (pH 7.4). The LDL protein was determined by bicinchoninic acid method using bovine serum albumin as a standard (Smith *et al.*, 1985).

Lipid peroxidation inhibitory assay

The assay for lipid peroxidation in human LDL was performed using the thiobarbituric acid (TBA) method as described previously (Wang *et al.*, 2004). Briefly, LDL (100 ㎍/㎖) was incubated with 5 μM Cu^{2+} or 5 mM AAPH in 0.2 ㎖ of 10 mM PBS buffer (pH 7.4) in the absence or presence of each compound at various concentration at 37 ℃ for 3 hrs. The oxidation was terminated by adding EDTA and BHT (1 mM and 50 μM final concentrations, respectively). Upon incu-

bation 0.2 mℓ aliquot was withdrawn and 0.5 mℓ TCA (20%) was added followed by 0.5 mℓ TBA (0.67%). The mixture was heated at 95-100 ℃ for 15 min, cooled in ice and centrifuged at 8000 rpm for 5 min. Then the absorbance of supernatant was read at 532 nm. Lipid peroxidation inhibitory activity (%) is expressed as follows:

Lipid peroxidation inhibitory activity (%) = [(Ac-As) / (Ac-Ab)]×100

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (without 5 μM Cu^{2+} or 5 mM AAPH). The IC_{50} value was defined as the concentration of sample required to inhibit 50% of thiobarbituric acid reactive substance (TBARS) formation.

RESULTS AND DISCUSSION

The MeOH extract of *C. sinensis* (the leaves) was suspended in H_2O and then partitioned successively with hexane, EtOAc, and BuOH. Among the partitioned fractions, the EtOAc-soluble fraction exhibited a significant antioxidant effect in a concentration-dependant manner with an IC_{50} value of 22.0 ± 2.5 μg/mℓ on DPPH assay (Table 1), suggesting that some compounds present in this fraction possess antioxidant effects. The subsequent bioassay-guided isolation of the EtOAc-soluble fraction led to the isolation of five known phenolic compounds (**1-5**), whose structures (Fig. 1) were identified as (+)-catechin (**1**) (Agrawal, 1989), kaempferol-3-*O*-α-L-rhamnopyranoside (**2**) (Fukunaga *et al.*, 1988), quercetin (**3**) (Zhong *et al.*, 1997), quercetin-3-*O*-α-L-rhamnopyranoside (**4**) (Kim *et al.*, 2004), and quercetin-3-*O*-β-D-glucopyranoside (**5**) (Choi *et al.*, 1998), respectively, by comparing their spectral (UV, IR, ^1H and ^{13}C-NMR, and ESI-MS) and physicochemical data with those reported in the literature.

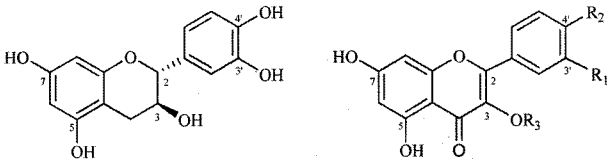
Flavonoids are generally known as effective antioxidants as well as radical scavengers (Rice-Evans *et al.*, 1997). Scavenging of free radicals seems to play a considerable part in the antioxidant ability of flavonoids. In order to understand the antioxidant behavior and the related activity-structure relationships of the flavonods (**1-5**) isolated from *C. sinensis*, it has been evaluated for their radical scavenging ability using DPPH and superoxide assays.

DPPH is a stable free radical and it has been widely used to test the free radical scavenging ability of phenolic compounds (Brand-Williams *et al.*, 1995). The radical scavenging effects of five flavonoids (**1-5**) on DPPH are shown in Table 2. The flavonoids (**1** and **3-5**) which possess an *o*-dihydroxy group in the B-ring exhibited considerable DPPH radical scavenging effects with IC_{50} values ranging from 21.3 ± 1.4 to 38.1 ± 3.2

Table 1. DPPH radical scavenging effects of the MeOH extract of *C. sinensis* and its solvent-fractions.

Extract/fractions	IC_{50} (μg/mℓ)†
MeOH extract	75 ± 3.6
Hexane fraction	> 100
EtOAc fraction	22.0 ± 2.5
BuOH fraction	69.3 ± 4.2
α-Tocopherol‡	21.8 ± 1.4

†The concentration of sample required to scavenge 50% of DPPH radicals.
‡The compound used as a positive control.
Data represented the mean ± SD of three individual experiments.



	R_1	R_2	R_3
2	H	OH	Rham
3	OH	OH	H
4	OH	OH	Rham
5	OH	OH	Glc

Fig. 1. Chemical structures of compounds **1-5** isolated from *C. sinensis*.

Table 2. Free radical scavenging effects of compounds **1-5** isolated from *C. sinensis* on DPPH and superoxide anion radicals.

Compounds	IC_{50} (μM)†	
	DPPH•	$•O_2^-$
1	33.9 ± 3.5	19.0 ± 2.8
2	> 100	> 100
3	21.3 ± 1.4	9.4 ± 0.7
4	38.1 ± 3.2	21.2 ± 3.6
5	36.1 ± 3.8	19.2 ± 2.9
α-Tocopherol‡	50.3 ± 1.5	–
Caffeic acid‡	41.2 ± 2.8	34.6 ± 3.7

†The concentration of sample required to scavenge 50% of free radicals.
‡The compounds used as positive controls.
Data represented the mean ± SD of three individual experiments.

μM in comparison with a positive control, α-tocopherol (IC_{50}: 50.3 ± 1.5 μM), but afzelin (**2**) which does not possess any *o*-dihydroxy group exhibited a weak radical scavenging effect (IC_{50}>100 μM). In addition, quercetin (**3**) which possesses a 2,3-double bond conjugated with a 4-oxo group in the C-ring exhibited a slightly effective scavenging effect than (+)-cat-

echin (**1**), whereas quercetin glycosides (**4** and **5**) exhibited weaker scavenging effects than (+)-catechin (**1**).

The superoxide anion radical ($\cdot O_2^-$) scavenging effects of the flavonoids (**1-5**) were investigated using the xanthine/xanthine oxidase system, and the results are presented in Table 2. Of these, flavonoids **1** and **3-5** exhibited considerable superoxide anion radical scavenging effects in a concentration-dependant manner with IC_{50} values ranging from 9.4 ± 0.7 to 21.2 ± 3.6 μM in comparison with a positive control, caffeic acid (IC_{50}: 34.6 ± 3.7 μM). The scavenging pattern was similar to that of DPPH assay.

Above two free radical scavenging results and literature (Bros *et al.*, 1990; Rice-Evans *et al.*, 1996; Arora *et al.*, 1998; Pietta, 2000; Burda & Oleszek, 2001) suggest that the structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of an *o*-dihydroxy (catechol) B-ring structure, which conferred higher stability in the radical form and participating in electron delocalization (Rice-Evans *et al.*, 1996; Pietta, 2000). Moreover, The 2,3-double bond conjugated with a 4-oxo group, which is responsible for electron delocalization from the B ring, enhances further the radical scavenging capacity (Bros *et al.*, 1990; Rice-Evans *et al.*, 1996), and saturation of the 2,3-double bond is believed to cause a loss of activity potential (Rice-Evans *et al.*, 1996; Arora *et al.*, 1998). Also, the free 3-OH group is important for their maximum radical scavenging potential (Burda & Oleszek, 2001), and substitution of the 3-OH group of flavonoids by a methyl or glycosyl group led to the loss in activity (Burda & Oleszek, 2001), probably due to steric effect (Bros *et al.*, 1990; Rice-Evans *et al.*, 1996).

Lipid peroxidation, specifically low-density lipoprotein (LDL) oxidation, appears to be a critical step in the pathogenesis of atherosclerotic vascular disease (Steinberg *et al.*, 1989; Aviram, 1993). Because flavonoids have been shown to be effective in the inhibition of LDL oxidation, many studies have been reported on their protecting effects on LDL oxidation. These effects may arise from the metal chelating and reactive oxygen radical scavenging ability of flavonoids. Therefore, the flavonoids (**1-5**) from *C. sinensis* were evaluated for their inhibitory effects on LDL peroxidation induced by Cu^{2+} or free radical generator AAPH using the TBA method, and the results are presented in Table 3. Again, flavonoids **1** and **3-5** exhibited considerable inhibitory effects on LDL peroxidation induced by either Cu^{2+} or AAPH with IC_{50} values ranging from 1.4 ± 0.4 to 11.9 ± 1.4 μM, confirming that the *o*-dihydroxy structure in the B ring is mostly responsible for metal chelating and free radical scavenging involved in lipid peroxidation of LDL (Brown *et al.*, 1998). However, all the flavonoids (**1-5**) tested were more effective at inhibiting the Cu^{2+}-induced peroxidation than the peroxidation induced by AAPH. This result suggested that both metal chelating and free radical scavenging mechanisms probably contributed to the inhibitory effects of the flavonoids on LDL peroxidation induced by Cu^{2+}. In the LDL peroxidation induced by AAPH, however, only the free radical scavenging mechanism contributed to the antioxidant action of the flavonoids, thereby accounting for the lower antioxidant effects (Arora *et al.*, 1998). Previous study demonstrated that flavonoids possessed good metal chelating ability, thereby suggesting that the metal chelating mechanism significantly contributed to the antioxidant potential of flavonoids (Morel *et al.*, 1993).

Table 3. Inhibitory effects of compounds **1-5** isolated from *C. sinensis* on LDL peroxidation induced by Cu^{2+} and AAPH.

Compounds	IC_{50} (μM)†	
	Cu^{2+}	AAPH
1	4.4 ± 0.1	9.8 ± 1.0
2	> 50	> 50
3	1.4 ± 0.4	9.1 ± 0.2
4	6.9 ± 1.2	11.9 ± 1.4
5	4.9 ± 1.1	10.8 ± 1.6
α-Tocopherol‡	29.9 ± 4.3	–
BHT‡	2.3 ± 0.2	10.1 ± 1.4

†The concentration of sample required to inhibit 50% of TBARS formation.
‡The compounds used as positive controls.
Data represented the mean ± SD of three individual experiments.

In conclusion, the results of this study indicate that flavonoids are the major constituents of *C. sinensis*, and among the isolated flavonods (**1-5**), (+)-catechin (**1**) and quercetin and its derivatives (**3-5**) play an important role in the revealing of a potent antioxidant effect of this plant. Therefore, *C. sinensis* is considered to be a good source of natural antioxidants as well as an effective nutritious food, whose consumption may contribute to better improved health and the prevention of oxidative stress-related diseases.

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LITERATURE CITED

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