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Antioxidant Flavonoids and Chlorogenic Acid from the Leaves of *Eriobotrya japonica*

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The antioxidant activity of *Eriobotrya japonica* was determined by measuring the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and lipid peroxidation produced when mouse liver homogenate was exposed to the air at 37°C, using 2-thiobarbituric acid (TBA). The methanol extract and its fractions of *Eriobotrya japonica* leaves showed strong antioxidant activity. The antioxidant activity of EtOAc and *n*-BuOH soluble fractions were stronger than the others, and were further purified by repeated silica gel, MCI gel CHP-20P, and Sephadex LH-20 column chromatography. Antioxidant chlorogenic acid, quercetin-3-sambubioside from *n*-BuOH fraction, and methyl chlorogenate, kaempferol- and quercetin-3-rhamnosides, together with the inactive ursolic acid and 2α-hydroxyursolic acid from EtOAc fraction were isolated. Antioxidant flavonoids and chlorogenic acid also showed prominent inhibitory activity against free radical generation in dichlorofluorescein (DCF) method.

Key words : *Eriobotrya japonica*, Antioxidant activity, Chlorogenic acid, Flavonoids

INTRODUCTION

Reactive oxygen species (ROS) in the forms superoxide, hydrogen peroxide, and hydroxyl radical are by-product of normal metabolism and attack biological molecules, leading to destabilization and disintegration of cell membranes, many age-related diseases, susceptibility to cancer (Yen and Chen, 1995; Osawa *et al.*, 1995). In addition, lipid peroxidation caused by these ROS is known as one of the major factors in the deterioration of food during storage and processing (Miyake *et al.*, 1997). Strong attention has recently been focused on the importance of protective defence systems in living cells against damage caused by active oxygen and free radicals. Several endogenous antioxidants such as vitamin E and vitamin C, have been found to play an important role in nonenzymatic protection. In addition, there is increasing interest in the protective biochemical function of dietary antioxidant. Recently, it has been reported that synthetic food antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are carcinogenic (Branen, 1975).

In previous paper, we screened different kinds of plants and marine algae in terms of their antioxidant effect on DPPH radical (Choi *et al.*, 1993) and reported that the methanolic extract of *Eriobotrya japonica* exhibited a strong antioxidant activity on DPPH radical.

The leaves of *Eriobotrya japonica* Lindl. (Rosaceae), a small tree commonly known as loquat, has been documented for use in folk medicine for the treatment of various skin diseases and diabetes mellitus (De Tommasi *et al.*, 1992). An alcoholic extract of the leaves has been shown to exhibit anti-inflammatory activity (Shimizu *et al.*, 1986), and a significant hypoglycemic effect in rabbits (Noreen *et al.*, 1988). An evaluation of hypoglycemic effects of sesquiterpene glycosides and triterpenoids isolated from *Eriobotrya japonica* has been reported (De Tommasi *et al.*, 1990; 1991; 1992). The methanolic extract and ursolic acid which is one of the major active compound from the leaves, showed strong antimutagenic and antitumor activities (Young *et al.*, 1994). However, the antioxidant activity of *Eriobotrya japonica* has not yet been investigated. In this paper, we report isolation of the components from the methanolic extracts of *Eriobotrya japonica* and describes their antioxidant effects on DPPH radical and their inhibitory effects of lipid peroxidation and free radical generation.

MATERIALS AND METHODS

IR spectra were recorded on Perkin Elmer FT-IR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were

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recorded at 300 MHz and 75.5 MHz respectively, on Bruker-AM 300 spectrometer with tetrametylsilane as the internal standard. FABMS spectra were obtained with a JMS-SX 102 mass spectrometer using a direct inlet system, and glycerol was used as a matrix. Multiplicities of ^{1}H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet), t (triplet), and m (multiplet).

Plant meterials

The leaves of *Eriobotrya japonica* were collected in 1996 at Sunchon, and authenticated by Professor J. C. Park of Department of Oriental Medicine Resourses, Sunchon National University.

Extraction and Isolation

Dried leaves (3.0 kg) were extracted three times with MeOH, upon removal of the solvent *in vacuo*, yielded a MeOH extract (400 g, 13.3% of dry weight). This MeOH extract was then suspended in H_2O and partitioned successively with CH_2Cl_2 (115 g), EtOAc (80 g), *n*-BuOH (110 g), and H_2O (90 g). The EtOAc and BuOH soluble fractions exhibiting strong antioxidant activity were further purified by repeated silica gel, MCI gel CHP-20P, and Sephadex LH-20 column chromatography. MCI gel CHP-20P column chromatography of the *n*-BuOH fraction was carried out, and 4 eluates, H_2O, H_2O-MeOH (3:2), H_2O-MeOH (2:3) and MeOH eluates, were obtained. Silica gel column (300 g, 4×50 cm, Merck) chromatography of H_2O-MeOH (3:2) eluate (4.0 g) was performed using $CHCl_3$-MeOH-H_2O (65:35:10, lower phase) as eluent. This fractionation resulted in nine fractions. Of these fractions, further separation of fractions 7 and 8 was carried out with Sephadex LH-20 column (50 g, 2×20 cm, Pharmacia, Sweden) chromatography using MeOH to yield quercetin 3-*O*-sambubioside (**2**, 250 mg) and chlorogenic acid (**1**, 380 mg), respectively. Sephadex LH-20 column (100 g, 4×50 cm) chromatography of the EtOAc fraction (35 g) was performed using MeOH and 4 subfractions were obtained. The fraction 1 was further chromatographed on a silica gel column (300 g, 4×50 cm, Merck) chromatography eluting with mixtures of hexane-EtOAc with increasing polarity (20:1→1:1) to yield ursolic acid (**7**, 1.5 g) and 2α-hydroxy ursolic acid (6, 0.8 g), respectively. The fraction 4 was further chromatographed on a silica gel column (300 g, 4×50 cm, Merck) chromatography eluting with mixtures of EtOAc-MeOH with increasing polarity (5:1→1:1). This fractionation resulted in six fractions. Of these fractions, further separation of fractions 4, 5, and 6 was carried out with Sephadex LH-20 column (50 g, 2×20 cm, Pharmacia, Sweden) chromatography using MeOH to yield afzelin (**4**, 40 mg), quercitrin (**5**, 150 mg) and methyl chlorogenic acid (**3**, 120 mg), respectively.

Compound 1 (Chlorogenic acid): White greyish powder from MeOH, blue fluorescence under uv light, IR ν_{max} (KBr, cm^{-1}); 3,368 (br,-OH), 1,686 (acid), 1,638 (α,β-unsaturated C=O), 1,610, 1,560 (aromatic), 1,442, 968 (*trans*), ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm; 9.57 (1H, s, 4'-OH), 9.15 (1H, s, 3'-OH), 7.43 (1H, d, *J*=16.11 Hz, H-7'), 7.04 (1H, d, *J*=1.17 Hz, H-2'), 6.99 (1H, dd, *J*=1.17 & 8.20 Hz, H-6'), 6.77 (1H, d, *J*=8.20 Hz, H-5'), 6.16 (1H, d, *J*=16.11 Hz, H-8'), 5.07 (1H, m, H-2 & H-6), ^{1}H-NMR (300 MHz, DMSO-d_6+D_2O) δ ppm; 7.46 (1H, d, *J*=15.4 Hz, H-7'), 7.09 (1H, s, H-2'), 7.06 (1H, d, *J*=8.1 Hz, H-6'), 6.83 (1H, d, *J*=8.1 Hz, H-5'), 6.23 (1H, d, *J*=15.4 Hz), 5.11 (1H, m H-3), 3.94 (1H, m, H-5), 3.63 (1H, m, H-4), 1.81~2.09 (4H, m, H-2 & H-6), ^{13}C-NMR (75.5 MHz, DMSO-d_6+D_2O) δ ppm; 174.85 (COOH), 165.71 (COO), 148.30 (C-4'), 145.53 (C-3'), 144.89 (C-7') 125.60 (C-1'), 121.31 (C-6'), 115.74 (C-2') 114.73 (C-5'), 114.31 (C-8'), 73.50 (C-1), 70.44 (C-5), 68.40 (C-4), 37.18 (C-6), 36.45 (C-2).

Compound 2 (Quercetin-3-sambubioside): Hygroscopic powder, Positive FABMS (*m/z*); 597[M+H]$^+$, 465 [M-$C_5O_4H_8$]$^+$, 303{M-$C_{11}O_9H_{18}$+H]$^+$, ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm; 12.66 (1H, brs. C5-OH), 7.72 (1H, dd, *J*=2.0 & 9.0, H-6') 7.59 (1H, d, *J*=2.0, H-2'), 6.89 (1H, d, *J*=9.0, H-5'), 6.91 (1H, d, *J*=2.0, H-8), 6.8 (1H, d, *J*=2.0, H-6), 5.72 (1H, d, *J*=7.0, anomeric), 4.76 (1H, d, *J*=7.1, anomeric), 3.1~3.8 (m), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ ppm; 177.3 (C=O), 164.1 (C-9), 161.2 (C-7), 156.2 (C-5), 155.2 (C-2), 148.5 (C-4'), 144.8 (C-3'), 133.0 (C-3), 122.2 (C-1'), 121.8 (C-2'), 121.1 (C-5'), 121.1 (C-6'), 116.0 (C-10), 115.8 (C-6), 115.2 (C-8), 98.5, 73.7, 77.6, 65.6, 76.8, 60.6 (glucose moiety), 104.4, 81.8, 76.1, 67.8, 59.9 (xylose moiety).

Compound 3 (Methyl chlorogenate): ^{1}H-NMR (300 MHz, DMSO-d_6+D_2O) δ ppm; 7.38 (1H, d, *J*=16.1 Hz, H-7'), 7.03 (1H, s, H-2'), 6.99 (1H, d, *J*=8.3 Hz, H-6'), 6.76 (1H, d, *J*=8.1 Hz, H-5'), 6.10 (1H, d, *J*=15.9 Hz), 5.02 (1H, m H-3), 3.56 (3H, s, $COOCH_3$), 3.33 (1H, m, H-5), 1.73~2.14 (4H, m, H-2 & H-6), ^{13}C-NMR (75.5 MHz, DMSO-d_6+D_2O) δ ppm; 173.60 (COOH), 165.35 (COO), 148.47 (C-4'), 145.61 (C-3'), 145.11 (C-7'), 125.35 (C-1'), 121.31 (C-6'), 115.83 (C-2') 114.59 (C-5'), 113.85 (C-8'), 73.03 (C-1), 70.85 (C-5), 69.32 (C-4), 37.24 (C-6), 35.08 (C-2), 51.87 ($COOCH_3$).

Compound 4 (Kaempferol-3-rhamnoside): ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm; 12.62 (1H, brs. C5-OH), 7.75 (2H, d, *J*=8.5, H-6' & H-2'), 6.92 (2H, d, *J*=8.8, H-5', H-3'), 6.42 (1H, d, *J*=2.0, H-8), 6.22 (1H, d, *J*=2.0, H-6), 5.30 (anomeric), 3.49~3.61 (m), 0.79 (3H, d, *J*=5.7, rha-CH_3), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ ppm; 177.7 (C=O), 164.3 (C-9), 160.0 (C-7), 157.2 (C-5), 156.5 (C-2), 134.2 (C-4'), 130.6 (C-3'), 131.5 (C-3), 130.8 (C-1'), 130.6 (C-2'), 130.6 (C-5'), 130.6 (C-6'), 120.5 (C-10), 115.4 (C-6), 115.6 (C-8), 101.8 (rha-C-1), 71.2, 70.6, 70.4, 70.0 (rhamnoside), 17.4 (rha-CH_3).

Compound 5 (Quercetin-3-rhamnoside): ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm; 12.65 (1H, brs. C5-OH), 7.29

(2H, d, J=8.5, H-6' & H-2'), 6.88 (1H, d, J=8.3, H-5'), 6.41 (1H, d, J=2.0, H-8), 6.22 (1H, d, J=2.0, H-6), 5.26 (1H, s, anomeric), 3.49~3.61 (m), 0.82 (3H, d, J=6.0, rha-CH_3), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ ppm; 177.7 (C=O), 164.4 (C-9), 161.3 (C-7), 157.2 (C-5), 156.4 (C-2), 148.5 (C-4'), 145.2 (C-3'), 134.1 (C-3), 121.1 (C-1'), 120.7 (C-2'), 115.7 (C-5'), 115.7 (C-6'), 115.6 (C-10), 115.5 (C-6), 115.0 (C-8), 101.0 (rha-C-1), 71.2, 70.6, 70.4, 70.0 (rhamnoside), 17.5 (rha-CH_3).

Compound 6 (2α-Hydroxy ursolic acid): ^{1}H-NMR (300 MHz, pyridine-d_5) δ ppm; 0.98 (3H, d, J=6.6 Hz, H-29), 0.99 (3H, s, H-25), 1.00 (3H, d, J=6.2 Hz, H-30), 1.01 (3H, s, H-24), 1.09 (3H, s, H-26), 1.22 (3H, s, H-27), 1.29 (3H, s, H-23), 2.64 (1H, d, J=10.8 Hz, H-18), 3.42 (1H, d, J=9.7 Hz, H-3α), 4.11 (1H, dt, J= 3.9, 9.7 Hz, H-2β), 5.47 (1H, t, J=3.5 Hz, H-12), ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ ppm; 179.77 (C-28, COOH), 139.21 (C-12), 125.49 (C-13), 83.73 (C-3), 68.53 (C-2), 55.87 (C-8), 39.77 (C-4), 39.44 (C-19), 39.36 (C-20), 38.39 (C-10), 37.37 (C-22), 33.47 (C-7), 31.04 (C-21), 29.31 (C-23), 28.59 (C-15), 24.84 (C-16), 23.88 (C-27), 23.69 (C-11), 21.38 (C-29), 18.79 (C-6), 17.63 (C-24), 17.47 (C-25), 17.41 (C-26), 16.97 (C-30).

Compound 7 (Ursolic acid): ^{1}H-NMR (300 MHz, pyridine-d_5) δ ppm; 0.91 (3H, d, J=6.6 Hz, H-29), 1.02 (3H, d, J=6.6 Hz, H-30), 1.05 (3H, s, H-24), 1.08 (3H, s, H-26), 1.25 (3H, s, H-27), 1.27 (3H, s, H-23), 2.66 (1H, d, J=3.5 Hz, H-18), 3.48 (1H, dd, J=10.3, 4.1Hz, H-3α), 5.52 (1H, t, J=3.5 Hz, H-12).

DPPH radical scavenging effect

Evaluation on the DPPH radical scavenging effect was carried out according to the method first employed by M. S. Blois (Blois, 1958). Four milliliters of MeOH solution of varying sample concentrations was added to 1.0 ml DPPH methanol solution (1.5×10^{-4}M). After standing at room temperature for 30 min., the absorbance of this solution was determined at 520 nm using a spectrophotometer and the remaining DPPH was calculated. The results were calculated by taking the mean of all triplicate values (Kim *et al.*, 1997).

In vitro lipid peroxidation test

Mice were sacrificed, and the liver removed. The liver was homogenized in 5ml of 0.9% NaCl and the homogenate volumed up 10 ml with 0.9% NaCl. A mixture of mouse liver homogenate (0.3 ml) and samples at various concentration (0.1 ml) in open test tube were incubated at 37°C. 1.5 ml of 20% acetic acid and 1 ml of 1.2% TBA solution were added. The test solution was heated for 30 min on boiling water bath, and then cooled at room temperature. The solution was centrifuged at 2500 rpm for 15 min, and the absorbance of upper layer was measurred at 532 nm (Ohkawa *et al.*, 1979; Igarashi and Ohmura, 1995). One unit of TBA value was determined when O.D. (optical density) of the absorbance was 0.1 at 532 nm (Han *et al.*, 1994).

Assay for the free radical generation

Liver cell (AC_2F) were incubated for 24 hrs. in serum free media in CO_2 incubator at 37°C until confluent, and the cells were transferred to multiwell plates with about 10^5 cells/well and cultured with or without a suspension of compounds 1-7 (10 μM), then incubated with 12.5 μM DCFH-DA at 37°C for 30 min. Fluorescence was monitored on a spectrofluorometer with excitation and emission wavelengths of 480 nm and 530 nm, respectively.

Statistics

Data were analyzed for statistical significance using student's *t*-test. Differences at a p value of less than 0. 05 were considered stastically significant.

RESULTS AND DISCUSSIONS

As indicated above, after screening of various plant extracts for their scavenging activity on DPPH radical (Choi *et al.*, 1993), a MeOH extract of *E. japonica* leaves was found to be the most potent at a concentration of 9.25 μg/ml. Therefore, the MeOH extract of *E. japonica* leaves further fractionated into CH_2Cl_2, EtOAc, BuOH and H_2O soluble fraction. As shown in Table I, the radical scavenging effects of the EtOAc and *n*-BuOH fractions were stronger than the others. Their SC_{50} were 2.76 μg/ml and 5.31 μg/ml, respectively. The results suggest that the MeOH extract, the EtOAc and *n*-BuOH fractions of *E. japonica* are effective radical scavengers. Therefore the EtOAc- and *n*-BuOH soluble fractions having strong antioxidant activity on DPPH radical were further purified by repeated silica gel, MCI gel CHP-20P and Sephadex LH-20 column chromatography. Compound **1** (chlorogenic acid) and compound **2** (quercetin-3-sambubioside) were isolated from *n*-BuOH fraction, and compound **3** (methyl chlorogenate), compound **4** (kaempferol-3-rhamnoside), compound **5** (quercetin-3-rhamnoside),

Table I. The radical scavenging effects of the MeOH extract and its subsequent fractions of *Eriobotrya japonica* leaves on DPPH radical

Samples	SC_{50} (μg/ml)[a]
MeOH ext.	9.25
CH_2Cl_2 fr.	29.1
EtOAc fr.	2.76
n-BuOH fr.	5.31
H_2O fr.	12.05
L-ascorbic acid	2.0

[a]50% scavenging concentration

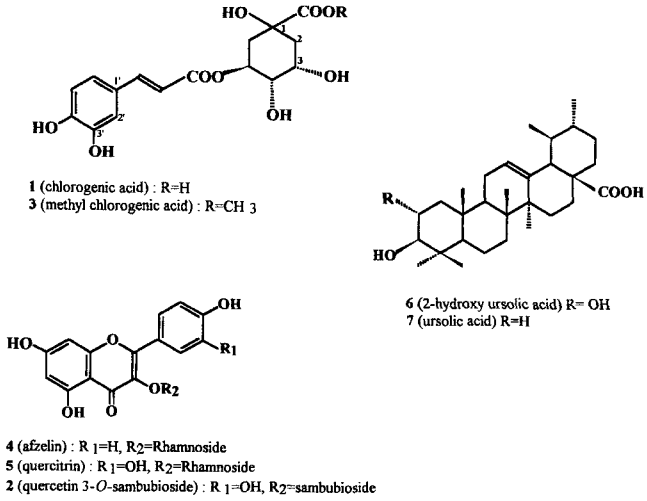

Fig. 1. Structures of *Eriobotrya japonica* components

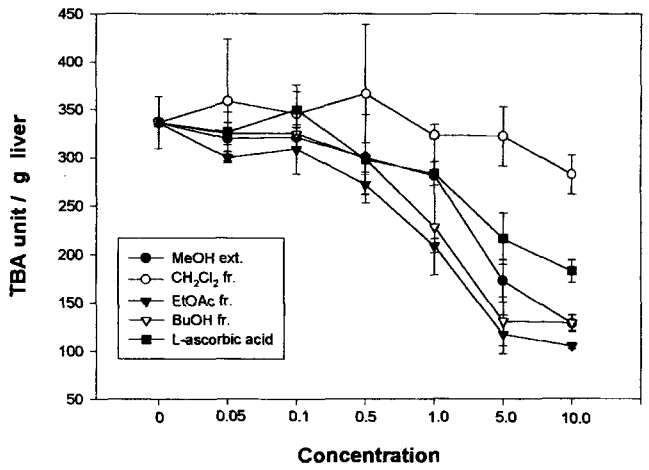

Fig. 2. The effect of various fractions obtained from the MeOH extract of *Eriobotrya japonica* on lipid peroxidation of liver homogenate. TBA values were measured after incubation for 6 hr.

compound **6** (2α-hydroxyursolic acid), and compound **7** (ursolic acid) from EtOAc fraction. Their structures (Fig. 1) were elucidated by the comparison of NMR spectral data with those reported in the literature (Young *et al.*, 1991, Mun *et al.*, 1994, Kim *et al.*, 1997 and Numata *et al.*, 1989). A report on their occurrence from the leaves of *E. japonica* has been unprecederted. Among these components, chlorogenic acid (**1**) and quercetin-3-sambubioside (**2**) exhibited higher scavenging activities on DPPH with SC_{50} of 6.35 μM and 9.62 μM, respectively (Table II). The antioxidant activities of two components were comparable to that of a strong antioxidant L-ascorbic acid. The higher radical scavenging property of chlorogenic acid and flavonoids are probably due to their structure (Mun *et al.*, 1994 and Kim *et al.*, 1997).

The antioxidant activity of *E. japonica* was determined by measuring lipid peroxides using TBA (Ohkawa *et al.*, 1979). TBA reacts with malondialdehyde (MDA), a product of lipid oxidation, to give a red, fluorescent 1 : 2 MDA/TBA adduct with maximum absorbance at 532 nm. MDA has been reported to inactivate ribonuclease and other enzymes in plant tissues and DNA in other systems (Du *et al.*, 1992). We measured auto-oxidation of lipid in mouse liver homogenate at different incubation time. Production of lipid peroxide reached a maximum in six hours (data not shown). Therefore, six hours were used to induce lipid peroxidation for further experiments in the present study. Fig. 2 shows the effects of various fractions obtained from the MeOH extract of *E. japonica* on lipid peroxidation of mouse liver homogenate. As shown in Fig. 2, MeOH extract, EtOAc and *n*-BuOH fractions inhibited the lipid peroxidation by 62%, 62% and 68%, respectively. But, CH_2Cl_2 fraction did not show the inhibitory activity of lipid peroxidation within the limit of tested concentration.

Table II. The radical scavenging effect of **1** and **2** from BuOH fraction on DPPH radical

Samples	SC_{50} (μM)[a]
1 (chlorogenic acid)	6.35
2 (quercetin-3-sambubioside)	9.62
L-ascorbic acid	14.36

[a]50% scavenging concentration

Recently, 2',7'-dichlorofluorescin diacetate (DCFH-DA) has been used as a probe of reactive oxygen species (ROS) such as $\cdot O_2^-$ and H_2O_2. Liposoluble DCFH-DA becomes water-soluble dichlorofluorescin (DCFH) as a results of the activities of mitochondrial esterase or hydrolysis, then it is oxidized to dichlorofluorescein (DCF) which has strong fluorescence. Therefore, this method is useful to measure changes of ROS (Label and Bondy, 1990). As shown in Table III, the MeOH extract of *E. japonica* leaves and their fractions except for CH_2Cl_2 fraction showed a significant inhibitory activity on free radical generation at concentrations of 0.5 mg/ml and 0.25 mg/ml, respectively. The inhibitory effects on free radical generation for the isolated compounds are shown in Table IV. Methyl chlorogenate (**3**) exhibited greater inhibitory action than the others (**1**, **2**, **4**, **5**) on the generation of free radicals of hepatocyte at the concentration of 10 μM. Wheares triterpene compounds such as 2α-hydroxyursolic acid (**6**) and ursolic acid (**7**) were found to be inactive at the same concentration. This indicated that structural modifications influence inhibition.

Chlorogenic acid is an ester of caffeic acid with quinic acid. Maruta *et al.* (1995) reported that caffeic acid derivatives were more effective than α-tocopherol in the *in vitro* hydroperoxidation of methyl linoleate, that the activity of caffeoylquinic acids on the molar

Table 3. The effect of the MeOH extract and its fractions of *E. japonica* leaves on free radical generation of hepatocyte (AC_2F)[a]

Samples	mean±S.E.	% of control
Control	14.00±0.40	
MeOH ext.	5.12±0.78***	36.55
CH_2Cl_2 fr.	10.97±1.58	78.33
EtOAc fr.	8.38±0.52**	59.82
n-BuOH fr.	7.87±0.32***	56.19
H_2O fr	4.04±0.35***	28.87

[a]Hepatocytes were incubated in serum free media and preparated at concentration of 0.5 mg/ml (MeOH) and 0.25 mg/ml (various fraction). After preincubation for 1 hr., 12.5 μM DCFH-DA were added and change in fluorescence was measured.
Values are means±S.E. of triplicate experiments.
Statistcal significance: *p<0.05, **p<0.01, ***p<0.001 vs control group.

basis increased in proportion to the number of caffeoyl residue and that esterification with quinic acid lowered the activity of caffeic acid. Also, chlorogenic acid and isochlorogenic acid were assessed as antioxidants in linoleic acid-aqueous system. In the mouse liver homogenate system, antioxidant activities of caffeic acid derivatives were also reported.

The antioxidative potency is related to the structure, in particular to electron delocalization of the aromatic nucleus. In fact, when these catechol flavonoids and chlorogenic acid react with the free radicals formed during autoxidation, they generate a new radical which is stabilized by the resonance effect of the aromatic nucleus (Torel *et al.*, 1986 and Cuvellier *et al.*, 1992).

The present study indicates that the methanol extract of *E. japonica* and their fractions, and their components, flavonoids and chlorogenic acids, may be useful for the treatment of oxidative damage. Investigation of further antioxidant principles is now in progress.

Table IV. The effect of compounds **1-7** from *E. japonica* leaves on free radical generation of hepatocyte (AC_2F)[a]

Compound	mean±S.E.	% of control
Control	9.00±0.20	
Chlorogenic acid (**1**)	6.41±0.30**	71.19
Quercetin-3-sambubioside (**2**)	6.70±0.26**	74.40
Methyl chlorogenate (**3**)	4.72±0.22***	52.44
Kaempferol-3-rhamnoside (**4**)	6.42±0.28**	71.38
Quercetin-3-rhamnoside (**5**)	6.00±0.08***	66.63
2α-hydroxyursolic acid (**6**)	8.44±0.34	93.71
Ursolic acid (**7**)	8.60±0.39	95.50

[a]Hepatocytes were incubated in serum free media and preparated at concentration of 10 μM. After preincubation for 1 hr., 12.5 μM DCFH-DA were added and change in fluorescence was measured.
Values are means±S.E. of triplicate experiments.
Statistcal significance: *p<0.05, **p<0.01, ***p<0.001 vs control group.

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