Arch. Pharm. Res. Vol.20, No.2, pp. 148-154, 1997

Chlorogenic Acid, an Antioxidant Principle from the Aerial Parts of *Artemisia iwayomogi* that Acts on 1,1-Diphenyl-2-picrylhydrazyl Radical

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(Received December 20, 1996)

The antioxidant activity of *Artemisia iwayomogi* was determined by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The methanol extract of *A. iwayomogi* showed strong antioxidant activity, and thus fractionated with several solvents. The antioxidant activity potential of the individual fraction was in the order of ethyl acetate > *n*-butanol > water > chloroform > *n*-hexane fraction. The ethyl acetate and *n*-butanol soluble fractions exhibiting strong antioxidant activity were further purified by repeated silica gel and Sephadex LH-20 column chromatography. Antioxidant chlorogenic acid was isolated as one of the active principles from the *n*-butanol fraction, together with the inactive components, 1-octacosanol, scopoletin, scopolin, apigenin 7,4'-di-*O*-methylether, luteolin 6,3'-di-*O*-methylether (jaceosidin), apigenin 7-methylether (genkwanin), 2,4-dihydroxy-6-methoxyacetophenone 4-*O*-β-D-glucopyranoside and quebrachitol. The antioxidant activity of chlorogenic acid was comparable to that of L-ascorbic acid, which is a well known antioxidant.

Key words : *Artemisia iwayomogi*, chlorogenic acid, antioxidant activity, 1-octacosanol, apigenin 7,4'-di-*O*-methylether, jaceosidin, genkwanin, 2,4-dihydroxy-6-methoxyacetophenone 4-*O*-β-D-glucopyranoside

INTRODUCTION

Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defence of living cells against oxidative damage. The toxic and otherwise unfavorable effects of synthesized food antioxidants have been widely noted. Nevertheless, phenolic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are still used extensively as food antioxidants because of their excellent results and low cost. When slightly larger doses (50 mg/kg/day) of these phenolic antioxidants are administered to rodents and monkeys, however, certain pathological, enzyme and lipid alterations as well as carcinogenic effects have been observed (Branen, 1975). The development of alternative natural antioxidants has, therefore, assumed an increased importance. Many investigators have found different types of antioxidants in various sources of plants (Larson, 1988).

In a previous paper, we reported results of screening tests on many plants and marine algae in terms of their antioxidant effect on 1,1,-diphenyl-2-picrylhydrazyl (DPPH) radical (Choi *et al.*, 1993). Among these samples, the methanolic extract of the aerial parts of *Artemisia iwayomogi* exerts a strong antioxidant activity on DPPH radical.

The aerial parts of *Artemisia iwayomogi* Kitamura (= *A. messer-schmidtiana* var. *viridis* Besser, Compositae) are used in Chinese herbal medicine as a choleretic, antiinflammatory, and diuretic agent in the treatment of epidemic hepatitis (Yook, 1989). Previous workers reported the isolation of esculetin 6-methylether, esculetin 7-methylether (scopoletin), scopolin, β-sitosterol, essential oil, fatty acid, sesquiterpene lactones, eudesmanolides and flavonoids (Hahn, 1966, Hahn and Kim, 1973, Greger *et al.*, 1986, Moon *et al.*, 1976, Valant-Vetschera and Wollenweber, 1995). The antimutagenic effect of the methanol extract of *A. iwayomogi* was demonstrated by us (Bae *et al.*, 1992).

In this paper, we now report isolation and structure elucidation of the components from the methanolic extract of *A. iwayomogi* and describes their antioxidant effects on DPPH radical.

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MATERIALS AND METHODS

Melting points were determined on a Electrothermal digital micro melting point apparatus and without correction. IR spectra were recorded on a Shimadzu IR-400 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded at 300 MHz and 75.5 MHz, respectively on a Bruker AM 300 spectrometer with tetramethylsilane as the internal standard. Multiplicities of ^{1}H- and ^{13}C-NMR signals are indicated as s (singlet), d (doublet) and t (triplet). The samples were run in DMSO-d_6, except for 1-octacosanol (**1**) and scopoletin (**2**), which were run in $CDCl_3$. UV spectra were run with Cecil 599 Universal automatic scanning spectrophotometer and electron impact mass spectra (EIMS) were taken on a Hewlett-Packard 5985B GC/MS spectrometer operating at 70 eV.

Plant materials

The aerial parts of *A. iwayomogi* were purchased from a commercial supplier in 1993, and authenticated by Prof. H. S. Young of the College of Pharmacy, Pusan National University. A voucher specimen has been deposited in the herbarium of the College of Pharmacy, Pusan National University.

Isolation of compounds

The powdered aerial parts (12.5 kg) of *A. iwayomogi* were extracted with MeOH and concentrated to give a dark residue (850 g), which was successively extracted with *n*-hexane (150 g), chloroform (30 g), ethyl acetate (70 g), *n*-butanol (240 g), and water (330 g). Each extract was tested for its scavenging effect on DPPH radical. The EtOAc (70 g) and *n*-BuOH (240 g) extracts exhibited strong scavenging activity on DPPH radical and so were subjected to silicagel column chromatography with EtOAc/MeOH mixtures, respectively. Compounds **1-5** were obtained from EtOAc extract and compounds **6-9** were obtained from *n*-BuOH extract, respectively.

Compound 1 (1-octacosanol): Colorless needles from MeOH, mp 83-85°C, IR ν_{max} (KBr, cm^{-1}) 3,410 (OH), 2,842 (CH), ^{1}H-NMR (300 MHz, $CDCl_3$) δ; 0.88 (3H, t, J=6.5 Hz, -CH_3), 1.26 (-CH_2), 1.55 (1H, -OH), 3.64 (2H, t, J=6.6 Hz, CH_2OH), ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ; 14.08 (-CH_3), 22.68, 25.79, 29.35, 29.45, 29.62, 29.70, 31.94, 32.85 (each, -CH_2), 63.12 (-CH_2OH), MS (*m/z*, %); 410[M]$^+$ (0.5), 392, 364, 336, 308, 266, 251, 181, 167, 153, 139, 125, 111, 97, 83 (100), 69

Compound 2 (scopoletin): Mp 204-5°C, IR ν_{max} (KBr, cm^{-1}); 3,340 (OH), 1,705 (α,β-unsaturated C=O), 1,610, 1,572, 1,510 (aromatic C=C), UV λ_{max} (log ε); in MeOH 229, 254, 261, 299, 346 nm, MS (*m/z*, %); 192 (M$^+$, 100), 177 (M$^+$-CH_3, 68), 164 (M$^+$-CO, 35), ^{1}H-NMR (300 MHz, $CDCl_3$) δ; 3.95 (3H, s, OCH_3), 6.26 (1H, d, J=9.5, H-3), 6.84 (1H, s, H-8), 6.92 (1H, s, H-5), 7.59 (1H, d, J=9.5, H-4), ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ; 56.41 (OMe), 166.32 (C-2), 113.40 (C-3), 107.51 (C-5), 143.25 (C-6), 149.24 (C-7), 103.49 (C-8), 149.24 (C-9), 113.40 (C-10)

Compound 3 (apigenin 7,4'-di-*O*-methylether): Colorless needles from MeOH, mp 120-121°C, MS (*m/z*, %); 298 (M$^+$, 8.0), ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 3.88 (3H, s, -OMe), 3.89 (3H, s, OCH_3), 6.48 (1H, d, J=2.0 Hz, H-6), 6.96 (1H, d, J=2.0 Hz, H-8), 7.04 (2H, d, J=9.0 Hz, H-2' and 6'), 7.84 (2H, d, J=9.0 Hz, H-3' and 5'), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 55.49 (OMe), 55.75 (OMe), 92.59 (C-8), 98.02 (C-6), 104.27 (C-3), 105.50 (C-10), 114.47 (C-3' and 5'), 124.50 (C-1'), 128.02 (C-2' and 6'), 157.67 (C-9), 162. 29 (C-4'), 162.70 (C-5), 164.01 (C-2), 165.42 (C-7), 182.99 (C-4)

Compound 4 (apigenin 7-methylether, genkwanin): mp 287-8°C, MS (*m/z*, %); 274 (M$^+$), ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 3.66 (3H, s, OCH_3), 6.35 (1H, d, J=2.0, H-8), 6.73 (1H, d, J=2.0, H-6), 6.79 (1H, s, H-3), 6.93 (2H, d, J=8.8, H-2' and 6'), 7.93 (2H, d, J= 8.8, H-3' and 5'), 12.93 (1H, brs, 5-OH), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 181.75 (C-4), 165.03 (C-7), 163.95 (C-2), 161.17 (C-4'), 161.09 (C-9), 157.18 (C-5), 128.42 (C-2' & 6'), 115.08 (C-1'), 115.84 (C-3' & 5'), 104.60 (C-10), 103.05 (C-3), 97.61 (C-6), 92.64 (C-8), 55.91 (OMe)

Compound 5 (luteolin 6,3'-di-*O*-methylether, jaceosidin): Mp 225-8°C, IR ν_{max} (KBr, cm^{-1}); 3,430 (OH), 1,635 (α,β-unsaturated C=O), 1,610, 1595, 1,565 (aromatic C=C), 1,500, 1,450, 1,350, 1,250, 1215, 1,155, 1,015, UV λmax (log ε); in MeOH 274 (4.29), 344 (4.48) nm; in NaOMe 262 (sh, 4.30), 274 (sh, 4.29), 335 (4.15), 408 (4.59); in NaOAc 276 (4.38), 325 (4.22), 363 (4.33); in NaOAc+H_3BO_3 275 (4.30), 344 (4.44); in $AlCl_3$ 262 (4.23), 280 (4.25), 298 (sh, 4.13), 377 (4.48); in $AlCl_3$+HCl 259 (4.19), 286 (4.26), 366 (4.45), ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 3.75 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.61 (1H, s, H-8), 6.88 (1H, s, H-3), 6.93 (1H, d, J=9.0, H-5'), 7.54 (1H, d, J= 9.0 and 2.0, H-6'), 7.55 (1H, d, J=2.0, H-2'), 13.07 (1H, brs, 5-OH), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 182.10 (C-4), 163.67 (C-2), 157.12 (C-7), 152.68 (C-5), 152.33 (C-9), 150.67 (C-4'), 147.97 (C-3'), 131.28 (C-6), 121.51 (C-1'), 120.28 (C-6'), 115.72 (C-5'), 110.21 (C-2'), 104.05 (C-10), 102.70 (C-3), 94.23 (C-8), 59.88 (OMe), 55.93 (OMe), MS (*m/z*, %); 330[M]$^+$ (27.9), 315[M-CH_3]$^+$ (18.2), 312[M-H_2O]$^+$ (13.2), 287[M-CH_3-CO]$^+$ (56.3), 272[M-2CH_3-CO]$^+$ (8.7), 183[A_1+H]$^+$, 7.9), 151[B_2]$^+$ (12.6), 148[B_1]$^+$ (10.8), 136[M-2CH_3-CO]$^+$ (16.9)

Compound 6 (2,4-dihydroxy 6-methoxy acetophenone 4-*O*-β-D-glucoside): Mp 156-8°C, IR (KBr, cm^{-1}); 3360, 2909, 1631, 1593, 1458, 1426, 1365, 1279, 1225, 1176, 1092, 1044, 824, UV λ_{max} (MeOH); 280.0, λ_{max} (MeOH+NaOMe); 282.0, ^{13}C-NMR (300 MHz, DMSO-d_6) δ; 202.83 ($CH_3\underline{CO}$), 165.16 (C-2)[a], 163.58

(C-4)[a], 162.54 (C-6)[a], 106.34 (C-1), 99.58 (Glc-1), 96.13 (C-3)[b], 91.92 (C-5)[b], 77.17 (Glc-3), 76.47 (Glc-5), 73.00 (Glc-2), 69.70 (Glc-4), 60.63 (Glc-6), 55.92 (Ar-OMe), 32.55 ($\underline{CH_3}CO$), a, b; interchangeable, ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 6.23 (1H, d, J=2.1 Hz, H-5), 6.15 (1H, d, J=2.1 Hz, H-3), 4.99 (1H, d, J=7.4 Hz, anomeric), 3.87 (3H, s, Ar-OMe), 3.12~3.73 (m, sugar-H), 2.58 (3H, s, -CH_3), MS (*m/z*, %); : 344 (M^+, 1.9), 182 (M^+-$C_6H_{10}O_5$, 86.4), 167 (182-CH_3, 100)

Compound 7 (scopolin): Colorless prisms from MeOH, mp 218°C, IR (cm^{-1}, KBr); 1,700 (α,β-unsaturated ketone), 1,500-1,600 (aromatic ring), 1,000-1,100 (glycoside), UV λ_{max} (MeOH); 213, 230, 254, 283, 340, ^{1}H-NMR (300 MHz, DMSO-d_6) δ; 3.16-3.73 (5H, m, H-2'~H-6'), 3.82 (3H, s, -OCH_3), 5.28 (1H, d, J=5.2 Hz, H-1'), 6.32 (1H, d, J=9.4 Hz, H-3), 7.16 (1H, s, H-8), 7.29 (1H, s, H-5), 7.96 (1H, d, J=9.4 Hz, H-4), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 56.03 (-OMe), 99.65 (C-1'), 73.32 (C-2'), 76.70 (C-3'), 69.59 (C-4'), 77.06 (C-5'), 60.62 (C-6'), 160.40 (C-2), 113.22 (C-3), 144.09 (C-4), 109.25 (C-5), 145.96 (C-6), 149.89 (C-7), 103.02 (C-8), 148.87 (C-9), 112.20 (C-10)

Compound 8 (chlorogenic acid): White greyish powder from MeOH, $FeCl_3$; dark green, mp 215-216°C, IR ν_{max} (KBr, cm^{-1}); 3,450 (br. -OH), 1,709 (acid), 1,642 (α,β-unsaturated C=O), 1,600, 1,534 (aromatic), 1,450, 980 (trans), UV λ_{max} nm; in MeOH; 222, 250, 304, 334, in MeOH+NaOH; 266, 312 (sh.), 380, ^{1}H-NMR (300 MHz, DMSO-d_6+D_2O) δ; 7.51 (1H, d, J=15.0 Hz, H-2'), 7.11 (1H, d, J=2.0 Hz, H-5'), 7.02 (1H, d, J=8.4 Hz, H-8'), 6.82 (1H, dd, J=2.0 & 8.4 Hz, H-9'), 6.33 (1H, d, J=15.0 Hz, H-3'), 5.21 (1H, dt, J=10.0 & 5.0 Hz, H-5), 4.09 (1H, m, H-3), 3.63 (1H, d, J=9.7 Hz, H-4), 1.87-2.00 (4H, m, H-2 & H-6), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 178.84 (C-7), 167.99 (C-1'), 148.90 (C-7'), 146.07 (C-2' & 8'), 126.61 (C-4'), 122.84 (C-9'), 116.74 (C-8'), 115.44 (C-5'), 115.09 (C-3'), 77.81 (C-1), 73.44 (C-3), 71.99 (C-4 & 5), 37.95 (C-2 & 6)

Acetylation of compound 8: A sample (30 mg) in pyridine and Ac_2O (1.5 ml, each) was allowed to stand at room temperature for 3 days. The reaction mixture was poured into crushed ice and filtered. The precipitate was crystallized from MeOH-H_2O to give white needles (35 mg), mp 143-5°C. IR ν_{max} (cm^{-1}); 1740, 1210 (acetate), MS (*m/z*, %); 180 ($C_9H_8O_4$, 39.5), 162 (180-H_2O, 100), Rf value (TLC, EtOAc:MeOH:H_2O=600:99:81); 0.20, ^{1}H-NMR (300 MHz, $CDCl_3$) δ; 7.60 (1H, d, J=16.0 Hz, H-2'), 7.42 (1H, dd, J=2.0 & 8.5 Hz, H-9'), 7.38 (1H, d, J=2.0 Hz, H-5'), 7.31 (1H, d, J=8.5 Hz, H-8'), 6.34 (1H, d, J=16.0 Hz, H-3'), 5.57 (1H, dt, J=10.0 & 4.1 Hz, H-5), 5.52 (1H, dd, J=4.1 & 10 Hz H-3), 5.12 (1H, dd, J=3.5 & 10 Hz, H-4), 2.72-2.39 (4H, m, H-2 & 6), 2.31, 2.30 (3H each, phenolic -OAc), 2.13, 2.07, 2.00 (3H each, aliphatic -OAc), ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ; 171.7 (C-7), 169.8, 169.6, 169.4, 167.7 (each, -$O\underline{C}OCH_3$), 165.0 (C-1'), 143.5 (C-7'), 142.2 (C-2' & 8'), 132.7 (C-4'), 126.3 (C-9'), 122.6 (C-8'), 118.2 (C-5'), 78.5 (C-1), 71.2 (C-3), 67.6 & 66.7 (C-4 or 5), 36.5 (C-2), 31.6 (C-6), 20.9, 20.7, 20.5 (each, -OCO$\underline{C}H_3$), 20.3 (-OCO$\underline{C}H_3$×2)

Compound 9 (quebrachitol): Colorless needles from MeOH, mp 191-196°C, IR (cm^{-1}, KBr); 3,316 (-OH), 2,922 (aliphatic CH), 1,377 (CH_3), 1,140, 1,051 (C-O), MS (*m/z*, %); 194 (M^+, 26), 158 (M^+-$2H_2O$, 0.5), 127, 87 (100), ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ; 57.00 (-OMe), 68.06 (C-1), 81.93 (C-2), 71.99 (C-3), 73.29 (C-4), 70.47 (C-5), 72.18 (C-6)

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.

RESULTS AND DISCUSSION

Structures of Isolated Compounds

Column chromatography on silica gel of the ethylacetate and *n*-butanol soluble fraction of the methanolic extract furnished compounds **1-5** and compounds **6-9** in the order of increasing polarity, respectively. Among them, compounds **2, 4, 7,** and **9** were readily elucidated as scopoletin, genkwanin, scopolin and quebrachitol respectively, by comparison of reported spectroscopic data and finally confirmed by comparison with authentic samples (Bae *et al.*, 1992, Zhang *et al.*, 1993, Valant-Vetschera and Wollenweber, 1995).

Compound **1**, mp 83~85°C, colorless needles crystallized from MeOH, showed a broad hydroxyl and CH stretching absorptions at 3,410 and 2,842 cm^{-1}, respectively. The ^{1}H-NMR spectrum of **1** in $CDCl_3$ showed characteristic signals for fatty alcohol; a triplet of three proton at δ 0.88 (J=6.5 Hz) and methylene of two protons at 1.26, a triplet of two protons at 3.64 (J=6.6 Hz) ascribable to secondary alcohol, a singet of one proton at 1.55 (OH). These data indicated that **1** was fatty alcohol derivatives. The mass spectrum of **1** showed the molecular ion peak at *m/z* 410 (0.5%). The physico-chemical and spectral data of **1** were identical with those of 1-octacosanol. The ^{13}C-NMR spectrum of **1** confirmed this suggestion. On the basis of these results, the structure of **1** was established as 1-octacosanol.

Compound **3**, mp 120~121°C, colorless needles crystallized from MeOH, showed two methoxy singlets at 3.88 and 3.89, a *meta*-coupled doublet of one proton at 6.48 and 6.96 (J=2.0, H-6 and H-8) and an *ortho*-coupled doublet of two proton at 7.04 and 7.84 (J=9.0, H-3',5' and H-2',6') in the ^{1}H-NMR spectrum. These data indicated that **3** was a 5,7,4'-oxygenated flavonoid derivatives. The mass spectrum of **3** showed the molecular ion peak at *m/z* 298 (35.0%) and *retro*-Diels-Alder fragmentation at *m/z* 168 (A ring+H, 8.8) and 132 (B ring, 12.4). The physicochemical and spectral data of **3** were identical with those of apigenin 7,4'-dimethylether. The ^{13}C-NMR spectrum of **3** confirmed this suggestion. On the basis of these results, the structure of **3** was established as apigenin 7,4'-dimethylether.

Compound **5**, mp 225~8°C, yellow needles crystallized from MeOH. Its IR spectrum showed a broad hydroxyl and α,β-unsaturated carbonyl absorptions at 3,430 and 1,665 cm^{-1}, respectively. The UV spectrum in MeOH showed absorption peaks characteristic of a flavone at 274 and 344 nm. A bathochromic shift of the UV with NaOMe, with an increase in intensity of band I, indicated the presence of a free 4'-hydroxyl group in **5**. And the UV spectrum also showed a bathochromic shift with $AlCl_3$ and $AlCl_3$+HCl in band I and with NaOAc in band II which indicated the presence of free 5-hydroxyl and 7-hydroxyl groups. The ^{1}H-NMR spectrum of **5** in DMSO-d_6 showed two methoxy singlets at δ3.75 and 3.88, a *meta*-coupled doublet of one proton at δ7.55 (J=2.0, H-2') and an *ortho*-coupled doublet of one proton at 6.93 (J=9.0, H-5'), a doublet of doublets of one proton at 7.54 (J=9.0 and 2.0, H-6'), two singlets of one proton at 6.61 (H-8) and 6.88 (H-3), and one proton at 13.07 (5-OH). These data indicated that **5** was a 5,6,7,3',4'-oxygenated flavonoid derivative. The mass spectrum of **5** showed the molecular ion peak at *m/z* 330 (27.9%) and *retro*-Diels-Alder fragmentation at *m/z* 183 (A ring+H, 7.9) and 148 (B ring, 10.8). The presence of intense peaks at *m/z* 315 ([M-CH_3]$^+$, 18.2), 312 ([M-H_2O]$^+$, 13.2), 287 ([M-CH_3-CO]$^+$, 56.3) and 272 ([M-2CH_3-CO]$^+$, 8.7) suggested that each of the methoxy groups was located at A ring and B ring, respectively. The strong intense peaks at *m/z* 315 and 287 suggested the presence of methoxy group of A ring at C-6. The physicochemical and spectral data of **5** were identical with those of 5,7,4'-trihydroxy 6,3'-dimethoxy flavone (jaceosidin). The ^{13}C-NMR spectrum of **5** confirmed this suggestion. On the basis of these results, the structure of **5** was established as 5,7,4'-trihydroxy 6,3'-dimethoxy flavone (jaceosidin) (Ulubelen *et al.*, 1977).

Compound **6**, mp 156~8°C, colorless needles crystallized from MeOH, which gave characteristic phenolic glycoside color reactions, greenish-brown in ferric chloride solution, and a positive Molisch test. The IR spectrum of **6** showed a broad hydroxyl and α,β-unsaturated carbonyl absorptions at 3,360 and 1,631 cm^{-1}, respectively. The UV spectrum in MeOH exhibited typical absorption maxima for acetophenone at 282 nm. The molecular ion peak at *m/z* 344 in the EI-MS was consistent with the molecular formula $C_{15}H_{20}O_9$. The ^{1}H-NMR spectrum of **6** in DMSO-d_6 showed the presence of acetyl (δ 2.58), a methoxyl (δ 3.87) group and a pair of *meta*-coupled aromatic protons (δ6.23, 6.15, J=2.1 Hz). It also showed the proton signals due to the sugar moieties between 3.12-4.99 including one anomeric proton signal (δ4.99, J=7.4 Hz). From acid hydrolysis of **6**, the sugar was identified as D-glucose by TLC. The configuration and conformation of D-glucose moiety was determined to be β-glucopyranose not only by the J value of the anomeric proton signal, but also by comparison of the ^{13}C-NMR data with those of corresponding methyl α-D- and β-D-glycosides. The glycosidic linkage site of β-D-glucopyranose and methoxyl group were determined to be C-4 and C-6, respectively, based on the spectral data comparison with those reported in the literature (Zhang *et al.*, 1993). On the basis of these results, the structure of **6** was established as 2,4-dihydroxy-6-methoxy acetophenone 4-*O*-β-D-glucopyranoside.

Compound **8**, mp 215~6°C, white greyish powder from MeOH, which gave characteristic phenolic color reactions, greenish-brown in ferric chloride solution. The IR spectrum of **8** showed a broad hydroxyl and α, β-unsaturated carbonyl absorptions at 3,450 and 1,642 cm^{-1}, respectively. The UV spectrum in MeOH exhibited typical absorption maxima for caffeic acid at 266 and 380 nm. Compound 8 gave a pentaacetate (**8a**), mp 143~5°C, on acetylation with Ac_2O/pyridine. The mass spectrum of **8a** did not show a molecular ion peak but two prominent ion peaks at *m/z* 180 ($C_9H_8O_4$, 39.5%) and 162 ($C_9H_8O_4$-H_2O, 100%) suggesting the presence of caffeoyl moiety. The NMR spectra of **8a** in $CDCl_3$ showed the signals of caffeoic acid (see Experimental) and two methylenes (36.5 and 31.6 ppm), one-oxygen bearing carbon (78.5 ppm) with acid (171.7 ppm) ascribable to cyclopolyoxycarboxylic acid, i.e. quinic acid. These spectral data were in agreement with those for the structure of caffeoyl quinic acid known as chlorogenic acid. The identity with chlorogenic acid was identified by comparison of NMR spectral data with those reported in the literature (Young *et al.*, 1991) and finally confirmed by direct comparison with an authentic sample.

The Radical Scavenging Effect of the Methanol Extract and Their Fractions of *A. iwayomogi* on DPPH Radical

Active oxygen species such as superoxide radicals, hydrogen peroxide and hydrogen radicals has been

recognized as the principle agent responsible for the deterioration of polyunsaturated fatty acids, or lipid containing foods when exposed to air (Slater *et al.*, 1987). The DPPH stable radical loses its characteristic purple color when supplied with electrons or hydrogen ions. The capacity of the tested substances to donate electrons can be estimated from the degree of their loss of color. The DPPH radical scavenging effect for the methanol extract and their fractions are shown in Table I. The radical scavenging effect for the methanol extract, and EtOAc and *n*-BuOH fractions obtained from the methanol extract was observed. The radical scavenging effect of the EtOAc and *n*-BuOH fractions were stronger than the others. Their IC_{50} were 8.2 μg/4 ml and 26.6 μg/4 ml, respectively. The results suggest that the methanol extract, and the EtOAc and *n*-BuOH fraction of *A. iwayomogi* are effective radical scavengers.

The Radical Scavenging Effect of Isolated Components on DPPH Radical

The radical scavenging effect of various components obtained from *A. iwayomogi* was shown in Table II. Among nine isolated compounds, chlorogenic acid isolated from the butanol fraction of the methanol extract of *A. iwayomogi* exhibited higher scavenging activity on DPPH with IC_{50} of 10.23 μM. The antioxidant activity of chlorogenic acid was comparable to those of L-ascorbic acid and BHT, which are well known antioxidant. These results suggest that the radical scavenging effect in the original methanol extract of *A. iwayomogi* was partially attributable to chlorogenic acid.

Chlorogenic acid, a naturally occurring polyphenolic compound, is reported as a clastogenic agent in hamster cells (Stich *et al.*, 1981) and to participate in enzymatic and nonenzymatic browing reactions in potatoes, sunflower seed, leaf protein concentrates, milk proteins, and other foods (Deshpande *et al.*, 1984). Chlorogenic acid also participates in the inhibition of 5-lipoxygenase activity in prostaglandin metabolism (Nishizawa *et al.*, 1988), inhibition of haematin-catalyzed retinoic acid 5,6-epoxidation (Iwahashi *et. al.*, 1986) and hepatoprotective effect in cultured rat hepatocytes against CCl_4-toxicity (Basnet *et al.*, 1996). Because chlorogenic acid has been reported to act as hepatoprotective agent, and *A. iwayomogi* also has a reputation in folklore regarding its use in the treatment of jaundice, in this study the isolation of active compound, chlorogenic acid from *A. iwayomogi* may validate this plant use in folklore against hepato-cellular damage. Other plants of this genus, such as *A. maritima* L. (Janbaz and Gilani, 1995), *A. scoparia* Thunb. (Gilani and Janbaz, 1993) and *A. absinthium* L. (Gilani and Janbaz, 1995) have recently been reported to show hepatoprotective activity.

Chlorogenic acid is an ester of caffeic acid with quinic acid. A comparison of the radical scavenging activity of caffeic acid and chlorogenic acid shows that esterification by quinic acid decreased the activity. Radical scavenging effect of phenolic compounds isolated from natural sources has been widely studied (Yoshida *et al.*, 1989). The antioxidative potency of phenolic acids are inter-related. These compounds react with the free radicals formed during autoxidation, and generate a new radical which is stabilized by the resonance effect of the aromatic nucleus (Cuvelier *et al.*, 1992). The higher radical scavenging property of catechol chlorogenic acid is probably due to a superior stability of radical derived from catechol compared to that of phenoxyl radical (Ruiz-Larrea *et al.*, 1994). The present work would tend to indicate that the methanol extract of *A. iwayomogi* and their fractions, and its component, chlorogenic acid, may be useful for the treatment of oxidative damage. Investigation of further antioxidant principles are now in progress.

Table I. Radical scavenging effects of the methanol extract of *A. iwayomogi* and their fractions on DPPH radical

Samples	IC_{50} (μg/4 ml)[a]
MeOH extract	15.2
Hexane fraction	275.4
$CHCl_3$ fraction	254.5
EtOAc fraction	8.2
BuOH fraction	26.6
H2O fraction	153.2
L-ascorbic acid	8.1
BHT[b]	9.5

[a]Amount required for reduction of DPPH radical after 30 min.
[b]Butylated hydroxytoluene.
Values are means of three experiments.

Table II. Radical scavenging effect of isolated compounds from *A. iwayomogi*

Samples	IC_{50} (μM)[a]
1-Octacosanol (**1**)	>320
Scopoletin (**2**)	225.65
Apigenin 7,4'-di-*O*-methylether (**3**)	>320
Genkwanin (**4**)	>320
Jaceosidin (**5**)	>320
2,4-Dihydroxy-6-methoxyacetophenone 4-*O*-β-D-glucopyranoside (**6**)	>320
Scopolin (**7**)	>320
Chlorogenic acid (**8**)	10.23
Quebrachitol (**9**)	>320
L-Ascorbic acid	9.15
Caffeic acid	5.24
Quinic acid	>320

[a]Amount required for reduction of DPPH radical after 30 min.
Values are means of three experiments.

ACKNOWLEDGEMENT

This work was supported in part by the G-7 project program.

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