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# Antioxidative Activity of Phenolic Acids Isolated from Jindalrae Flower (*Rhododendron mucronulatum* Turzaninow)

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**Abstract** : Six phenolic acids were isolated from Jindalrae flowers (*Rhododendron mucronulatum* Turcz.), an edible plant in Korea. These compounds were identified as chlorogenic acid, 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid, caffeic acid, ferulic acid, and p-coumaric acid on the basis of IR, UV, $^{1}H$ and $^{13}C$ NMR, FAB-MS, ES-MS and/or EI-MS data. Chlorogenic acid (0.2 g) present in both ethyl acetate and ethyl ether fractions comprised up to 38.5% of the total phenolic acid amount (0.52 g) finally recovered by means of polyamide C-200 column chromatography, preparative TLC, recrystallization, and Sephadex LH-20 column chromatography. The antioxidant activities were measured in an ethanol solution of linoleic acid in the presence of ferric thiocyanate. The antioxidant efficiency increased in the order of p-coumaric acid<α-tocopherol <ferulic acid<caffeic acid<chlorogenic acid<4,5-dicaffeoylquinic acid<3,5-dicaffeoylquinic acid.(Received August 22, 1996; accepted October 2, 1996)

## Introduction

Phenolic acids have been implicated as possibly influencing the toxicological, nutritional, colour, sensory and antioxidant properties of foods with which they are associated.[1)] They have been shown to be directly involved in the biosynthesis of coumarins, flavonoids and lignin.[2)] Hydroxycinnamic acids, a major class of phenolic acids, occur naturally as either free or in a wide range of bound or conjugated forms containing organic acids, sugars, amino compounds, lipids, terpenoids and other phenolics. Hydroxycinnamic acids and their derivatives are almost exclusively derived from p-coumaric, caffeic, and ferulic acid, whereas sinapic acid is comparatively rare.[3)] Hydroxycinnamic acids usually occur in various conjugated forms, more frequently as esters than glycosides. Although phenolic acids are present both in the plant and animal world, most of them are of plant origin.[4)]

There are some 1900 extant species in the Ericaceae family. Of these, 30 species including Jindalrae (*Rhododendron mucronulatum* Turzaninow), Cheolchchug (*Rhododendron schlipenbachii* Max.), Sancheolchchug (*Rhododendron poukhanense* Leveil), Manbyeongcho (*Rhododendron brachycarpum D. Don. var. typicum* Nakai), and so forth are widely distributed in Korea.[5)] Jindalrae is commonly cultivated as an ornamental plant or used as a folk remedy for diarrhoea, diuresis, emesis, eruption, and rheumatism in Korea.[6)] Jindalrae flowers have also been used for raw materials of colorful Korean traditional dishes. We found that acidic fractions obtained from both ethyl acetate and ethyl ether fractions of Jindalrae flowers showed a significant antioxidant activity in an *in vitro* fatty acid peroxidation assay. For this reason, we have started in an investigation concerning antioxidants. Although many of flavonoids and simple phenols in the leaves of the other members of *Rhododendron* were already investigated,[7,8)] no investigation has been made on phenolic acids of Jindalrae flowers. In the course of our investigasion on antioxidative constituents, we isolated and characterized six phenolic acids as antioxidative principles in Jindalrae flowers.

## Materials and Methods

### General Procedure and Materials

Jindalrae flowers were collected at Tongyoung, Kyoungsangnam-do, Korea, in April 1993. The extracting and developing solvents, and polyamide C-200 for column chromatography were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and reagents for antioxidant assay, then, from Aldrich Chemical Co. (Milwaukee, WI, USA). The authentic phenolic acids were obtained from Sigma Chemical Co. (St. Louis, Mo, USA).

Key words : Antioxidants; phenolic acids; Jindalrae; Korean azalea flower; *Rhododendron mucronulatum*; Ericaceae
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Melting points were measured on a Büchi 535 melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-5000. $^1H$ and $^{13}C$ Nuclear magnetic resonance (NMR) spectra were measured 500 and 125 MHz, respectively, on a Bruker AM-500. Fast atom bombardment mass spectrometry (FAB-MS) was carried out with a VG ZAB-2F and electrospray mass spectrometry (ES-MS) on a VG Quattro-BQ and electron-impact mass spectrometry (EI-MS) on a VG Trio-2. UV/visible spectra were recorded on a Shimadzu UV-2100.

### Extraction and Isolation

Jindalrae flowers (20.5 kg) were extracted twice with 40 L of 80% MeOH and of 50% MeOH at room temperature, respectively. The aqueous MeOH extracts were combined and concentrated under the reduced pressure to remove most of MeOH. The remaining aqueous solution was successively partitioned with n-hexane, ethyl ether, and ethyl acetate, resulting in four fractions of differing polarity, namely, n-hexane, ethyl ether, ethyl acetate, and water fraction. Of these, the ethyl ether and ethyl acetate fractions were extracted with 5% sodium bicarbonate solution and subsequently adjusted pH 1.0 with 5% hydrochloric acid, respectively. The resulting acidic fractions were extracted three times with the corresponding solvents, repectively. The ethyl ether (1.15 g) and ethyl acetate (9.26 g) solutions containing acidic compounds were dried over anhydrous sodium sulfate, filtered, and freed of solvent under reduced pressure.

Total of the ethyl ether (A) and ethyl acetate (B) fractions were adsorbed onto polyamide C-200 (4.5×50 cm) and continuously eluted by using $CHCl_3$-MeOH-$H_2O$ (30 : 10 : 1) to provide seven fractions (300 m*l*), respectively. The fractions were spotted on TLC plates precoated with silica gel 60 $F_{254}$ (particle size, 250 μm, Merck). The plates were developed with EtOAc-$H_2O$-HCOOH (10 : 9 : 1, system 1) or $CHCl_3$-MeOH-HOAc (80 : 20 : 1, system 2) mixture and then completely air-dried in a draft chamber. Phenolic acid zones (compound **1**, **2** and **3** from fr. B-4 and 5, compound **4**, **5** and **6** from fr. A-2 and 3) separated on the plates were detected under UV light (350 nm) without and with ammonia vapour (UV/$NH_3$) and subsequently scraped off with a spatula. Each scraped-off zone (UV : blue or blue-violet, UV/$NH_3$ : blue-green or violet) was placed in a 300 m*l* round bottom flask containing an adequate amount of MeOH and gently heated for 20 min, occasionally swirling it. Subsequently, the hot mixture was filtered on a Büchner funnel, followed by washing with MeOH. The resulting filterate was subsequently concentrated to one-third of its original volume *in vacuo* with a rotary evaporator and precipitated by adding a small amount of nonpolar solvent such as chloroform, followed by filteration on a Büchner funnel. The precipitate was washed with cold-MeOH, followed by purification on a Sephadex LH-20 (3×25 cm, Pharmacia Biotech., Sweden) column chromatography using MeOH-$H_2O$ (4 : 1) as an eluting solution.

### Antioxidative Assay in Solution

Each sample dissolved in 120 μ*l* of EtOH was added to a reaction mixture in a screw-cap test tube. The reaction mixture consisted of 2.88 m*l* of 2.51% linoleic acid in EtOH and 9 m*l* of 40 mM phosphate buffer (pH 7.0). The test tube was placed in an incubator at 40°C. At daily intervals during an incubation period, a 0.1 m*l* aliquot of the mixture was diluted with 9.7 m*l* of 75% EtOH, followed by adding 0.1 m*l* of 30% ammonium thiocyanate. Precisely, 3 min after the addition of 0.1 m*l* of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm.[9]

### Spectral Characteristics of Compound 1 through 6

Compound **1** (0.2 g) : White powder; mp 211~213°C; IR $\nu_{max}^{KBr}$ cm $^{-1}$ 3470~3357 (OH), 2927, 1727 (>C=O, ester), 1687 (>C=O, carboxylic acid), 1601 (aromatic), 1458 (cycloalkane), 1189 (phenol), 978 (trans alkene); UV $\lambda_{max}^{MeOH}$ nm 216, 242, 300 (sh), 326; TLC (system 1) $R_f$ 0.21; FAB-MS *m/z* 355 $[M+H]^+$; $^1H$ NMR ($CD_3OD$) δ 7.58 (1H, *d*, *J*=15.9, H-7'), 7.07 (1H, *d*, *J*=2.0, H-2'), 6.96 (1H, *dd*, *J*=8.2, 2.0, H-6'), 6.81 (1H, *d*, *J*=8.2, H-5'), 6.29 (1H, *d*, *J*=15.9, H-8'), 5.36 (1H, *ddd*, *J*=9.3, 8.5, 4.5, H-5), 4.20 (1H, *ddd*, *J*=3.1, 4.4, 3.1, H-3), 3.76 (1H, *dd*, *J*=8.5, 3.1, H-4), 2.26 (1H, *dd*, *J*=13.3, 4.5, H-6(eq)), 2.19 (1H, *dd*, *J*=13.3, 3.1, H-2(ax)), 2.10 (1H, *dd*, *J*=13.3, 9.3, H-6(ax)), 2.07 (1H, *dd*, *J*=13.3, 4.5, H-2(eq)); $^{13}C$ NMR ($CD_3OD$) δ 177.3 (*s*, C-7), 169.0 (*s*, C-9'), 149.8 (*s*, C-4'), 147.4 (*d*, C-7'), 147.0 (*s*, C-3'), 128.0 (*s*, C-1'), 123.3 (*d*, C-6'), 116.8 (*d*, C-8'), 115.5 (2×*d*, C-2' & C-5'), 76.4 (*s*, C-1), 73.7 (*d*, C-4), 72.2 (*d*, C-5), 71.6 (*d*, C-3), 39.0 (*t*, C-6), 38.4(*t*, C-2).

Compound **2** (0.16 g) : White powder; mp 170~172°C (dec.); IR $\nu_{max}^{KBr}$ cm$^{-1}$ 3400~3200 (OH), 1730 (>C=O, ester), 1703 (>C=O, carboxylic acid), 1675 (trans alkene), 1475 (cycloalkane), 1202 (phenol), 830 (aromatic); UV $\lambda_{max}^{MeOH}$ nm 250 (sh), 300 (sh), 331; TLC (system 1) $R_f$ 0.60; FAB-MS *m/z* 517 $[M+H]^+$; $^1H$ NMR ($CD_3OD$) δ 7.62 (1H, *d*, *J*=15.8, H-7' or H-7"), 7.58 (1H, *d*, *J*=15.8, H-7' or H-7"), 7.07 (1H, *d*, *J*=2.0, H-2' or H-2"), 7,06 (1H, *d*, *J*=2.0, H-2' or H-2"), 6.98 (1H, *dd*, *J*=8.2, 2.0, H-6' or H-6"), 6.96 (1H, *dd*, *J*=8.2, 2.0, H-6' or H-6"), 6.79 (1H, *d*, *J*=8.2, H-5' or H-5"), 6.78 (1H, *d*, *J*=8.2, H-5' or H-5"), 6.34 (1H, *d*, *J*=15.8, H-8' or 8"), 6.25 (1H, *d*, *J*=15.8, H-8' or H-8"), 5.48 (1H, *m*, H-3), 5.39 (1H, *m*, H-5), 3.98 (1H, *dd*, *J*=6.6, 3.0, H-4), 2.10~2.36 (4H,

*m*, 2 × H-2 & H-6); $^{13}C$ NMR ($CD_3OD$) δ 177.5 (*s*, C-7), 168.8 (*s*, C-9' or C-9"), 168.0 (*s*, C-9' or C-9"), 149.8 (*s*, C-3' or C-3"), 149.6 (*s*, C-3' or C-3"), 147.5 (*d*, C-7' or C-7"), 147.2 (*s*, C-4' or C-4"), 146.9 (*s*, C-4' or C-4"), 146.8 (*d*, C-7' or C-7"), 127.9 (*s*, C-1' or C-1"), 127.6 (*s*, C-1' or C-1"), 123.1 (*d*, C-6' or C-6"), 123.0 (*d*, C-6' or C-6"), 116.6 (*d*, C-5' or C-5"), 116.5 (*d*, C-5' or C-5"), 115.5 (*d*, C-8' or C-8"), 115.2 (2×*d*, C-2' & C-2"), 114.9 (*d*, C-8' or C-8"), 76.6 (*s*, C-1), 72.3 (*d*, C-3), 72.0 (*d*, C-5), 69.7 (*d*, C-4), 38.6 (*t*, C-2), 37.6 (*t*, C-6).

Compound **3** (0.1 g) : White powder; mp 142℃ (dec.); IR $\nu_{max}^{KBr}$ $cm^{-1}$ 3450~3230 (OH), 1733 (>C=O, ester), 1710 (>C=O, carboxylic acid), 1670 (trans alkene), 1468 (cycloalkane), 1208 (phenol), 840 (aromatic); UV $\lambda_{max}^{MeOH}$ nm 250 (sh), 300 (sh), 332; TLC (system 1) $R_f$ 0.38; FAB-MS *m/z* 517 $[M+H]^+$; $^1H$ NMR ($CD_3OD$) δ 7.60 (1H, *d*, *J*=15.8, H-7' or H-7"), 7.52 (1H, *d*, *J*=15.8, H-7' or H-7"), 7.09 (1H, *J*=2.0, H-2' or 2"), 7.07 (1H, *J*=2.0, H-2' or H-2"), 6.99 (1H, *dd*, *J*=8.2, 2.0, H-6' or H-6"), 6.97 (1H, *dd*, *J*=8.2, 2.0, H-6' or 6"), 6.80 (1H, *d*, *J*=8.2, H-5' or H-5"), 6.77 (1H, *d*, *J*=8.2, H-5' or H-5"), 6.29 (1H, *d*, *J*=15.8, H-8' or H-8"), 6.20 (1H, *d*, *J*=15.8, H-8' or H-8"), 5.67 (1H, *m*, H-5), 5.15 (1H, *dd*, *J*=8.0, 3.3, H-4), 4.36 (1H, *m*, H-3), 2.00~2.30 (4H, *m*, 2×H-2 & H-6); $^{13}C$ NMR ($CD_3OD$) δ 177.0 (*s*, C-7), 168.5 (*s*, C-9' or C-9"), 167.9 (*s*, C-9' or C-9"), 149.8 (2×*s*, C-3' & 3"), 147.8 (*d*, C-7' or C-7"), 147.5 (*s*, C-4' or C-4"), 147.2 (*s*, C-4' or C-4"), 146.9 (*d*, C-7' or C-7"), 127.7 (*s*, C-1' or C-1"), 127.5 (*s*, C-1' or 1"), 123.2 (*d*, C-6' or C-6"), 116.6 (*d*, C-5' or C-5"), 116.5 (*d*, C-5' or C-5"), 115.2 (2×*d*, C-2' & C-2"), 114.7 (*d*, C-8' or C-8"), 114.5 (*d*, C-8' or C-8"), 76.2 (*s*, C-1), 74.9 (*d*, C-4), 69.1 (*d*, C-5), 68.6 (*d*, C-3), 38.8 (*t*, C-6), 38.4 (*t*, C-2).

Compound **4** (0.03 g) : Yellow powder; mp 223~225℃ (dec.); TLC (system 2) $R_f$ 0.58; EI-MS *m/z* 180. The other spectral data were similar to those of the cited references.[10,11]

Compound **5** (0.01 g) : White powder; mp 168~171℃; TLC (system 2) $R_f$ 0.81; EI-MS *m/z* 194. The other spectral data were similar to those of the cited references.[10,11]

Compound **6** (0.02 g) : White powder; mp 210~213℃; TLC (system 2) $R_f$ 0.65; EI-MS *m/z* 164. The other spectral data were similar to those of the cited references.[10,11]

## Results and Discussion

### Structural Analysis of Compounds 1 through 6

In the present study, six phenolic acids isolated from Jindalrae flowers were confirmed as 5-*O*-caffeoylquinic acid (chlorogenic acid), 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid, 3,4-dihydroxycinnamic acid (caffeic acid), 4-hydroxy-3-methoxycinnamic acid (ferulic acid) and p-hydroxycinnamic acid (p-coumaric acid) by a detailed comparison of their IR, UV, MS, and $^1H$ and $^{13}C$ NMR spectral characteristics. Compound **1**, **2** and **3** were quinyl esters of caffeic acid. The other compounds were cinnamic acid derivatives.

The IR spectral data of compound **1** suggested the presence of a caffeoyl moiety and a quinic acid. Its $^1H$ NMR spectrum also showed three aromatic protons of a ABX system, indicating the presence of 1,2,4-trisubstituted benzene and two trans olefinic protons (δ 7.58 and 6.29, *J*=15.9 Hz) due to a caffeoyl moiety. The signals of H-3 (equatorial), H-4 (axial), and H-5 (axial) of the quinic acid moiety were assigned according to their multiplicity and their spin-spin coupling constants ($J_{3,4}$= 3.1, $J_{4,5}$=8.5, $J_{5,6eq}$=4.5, and $J_{5,6ax}$=9.3 Hz). Furthermore, a detailed comparison of its $^{13}C$ NMR with that of caffeic acid mostly confirmed the signals due to a caffeoyl moiety, establishing the structure of **1** as a caffeic acid derivative. In confirmation of the presence of a quinic acid moiety, negative ion ES-MS were more informative than the other spectral data. This mass spectrum showed an intense quasimolecular ion peak $[M-H]^-$ of **1** at *m/z* 353 and that of quinic acid at *m/z* 191, suggesting the elimination of a caffeoyl moiety from the original molecular weight. Since all of the spectral data were identical with the structure, this compound was confirmed as 5-*O*-caffeoylquinic acid (chlorogenic acid).

The negative ion ES-MS spectrum of compound **2** indicated a quasimolecular ion peak at *m/z* 515 and that of quinic acid at *m/z* 191, suggesting the loss of two caffeoyl moieties from its original molecular weight. Its $^1H$ and $^{13}C$ NMR data also showed the presence of two caffeoyl moieties and a quinic acid moiety in the molecular structure. The $^1H$ NMR data were mostly similar to those of **1** except for the chemical shift of H-3 (δ 5.48) of its esterification site. In the case of **2**, the signal of H-3 of the quinic acid moiety was shifted downfield by 1.28 ppm as compared with that of chlorogenic acid. The $^{13}C$ NMR data similarly showed the downfield shift by 0.7 ppm at C-3 as compared with that of compound **1**. Consequently, this compound was confirmed as 3,5-*O*-dicaffeoylquinic acid having the molecular formula of $C_{25}H_{24}O_{12}$.

The positive ion ES-MS spectrum of compound **3** showed a quasimolecular ion peak at *m/z* 517, two caffeoyl moieties at *m/z* 326, a quasimolecular ion peak of quinic acid at *m/z* 193, and a caffeoyl moiety at *m/z* 163, respectively. Its $^1H$ and $^{13}C$ NMR data also indicated that the molecular structure is consisted of two caffeoyl moieties and a quinic acid. On the other hand, these NMR data were mostly similar to those of **1** except for the chemical shifts of H-4 (δ 5.15) and C-4 (δ 74.9), suggesting the presence of the esterified position. On the basis of various spectral data explained above, this

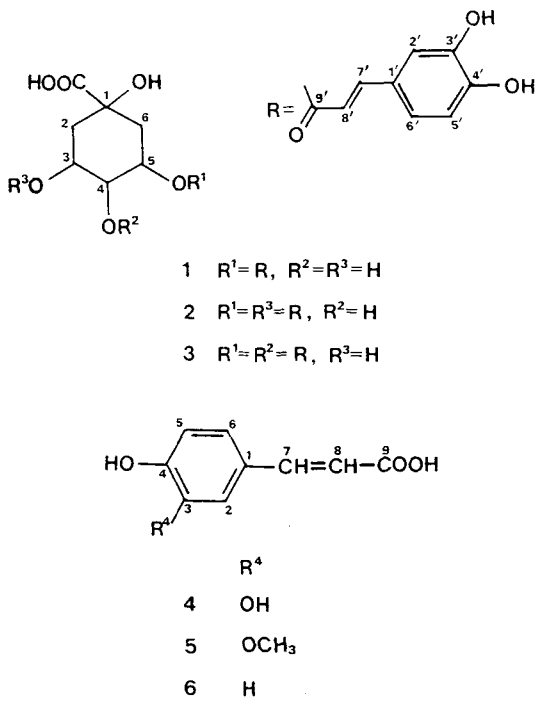


Fig. 1. Chemical structures of phenolic acids isolated from Jindalrae flower.

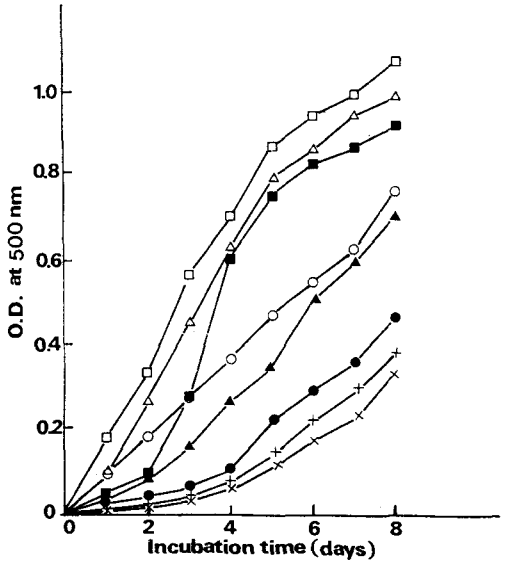


Fig. 2. Antioxidative activity of phenolic acids isolated from Jindalrae flower on α-tocopherol at the level of 10 ppm. (×—×, 3,5-*O*-dicaffeoylquinic acid; +—+, 4,5-*O*-dicaffeoylquinic acid; ●—●, chlorogenic acid; ▲—▲, caffeic acid; ○—○, ferulic acid; ■—■, α-tocopherol(10 ppm); △—△, p-coumaric acid; □—□, control).

compound was identified as 4,5-*O*-dicaffeoylquinic acid having the molecular formula of $C_{25}H_{24}O_{12}$.

The IR spectral data of compound **4** suggested the presence of an aromatic ring, a trans olefinic group, a carboxyl group and hydroxyl group. Its mass fragmentation obtained by EI-MS supported the results obtained from the IR spectrum. The $^1H$ NMR data showed the presence of three aromatic protons (a ABX system) and two trans olefinic protons. The $^{13}C$ NMR data indicated the signals for two carbons of an olefinyl moiety, one carbon of a carboxyl group and six carbons of an aromatic ring. Therefore, compound **4** was confirmed as caffeic acid to be $C_9H_8O_4$.

Compound **5** showed its molecular ion peak at *m/z* 194 by EI-MS. EI-MS spectral data of **5** were as follows: *m/z* (%) 194 (85, $M^+$), 179 (68, $M^+$-$CH_3$), 177 (62, $M^+$-OH), 166 (18, $M^+$-$CH_2$=$CH_2$), 161 (40, $M^+$-$CH_3$-$H_2O$), 150 (82, $M^+$-$CO_2$), 149 (52, $M^+$-COOH), 145 (55, $M^+$-$H_2O$-$CH_3O$·), 135 (79, $M^+$-$CO_2$-$CH_3$·), 133 (68, $M^+$-$CH_3$-HCOOH), 123 (57, $M^+$-CH=CHCOOH), 117 (56, $M^+$-$C_6H_5$·), 107 (78, $M^+$-CH≡CH-$CO_2$-OH), 105 (67, $M^+$-$CH_2$=$CH_2$-$CO_2$-OH), 95 (66, $M^+$-$CH_2$=$CH_2$-CH=CHCOOH), 91 (58, $C_6H_5CH_2$·), 89 (94, $CH_2$=$CH_2$+$CO_2$+OH), 77 (82, $C_6H_5$·), 67 (78, $C_5H_6$+H·), 53 (100, $C_4H_4$+H·), 45 (59, ·COOH). These cleavage patterns suggested the presence of a hydroxy-methoxyphenyl ring, a olefinic moiety, and a carboxyl group. These mass spectral data supported the characteristic absorption bands recognized on the IR spectrum. The $^1H$ and $^{13}C$ NMR data also suggested the presence of a 4-hydroxy-3-methoxyphenyl ring, a trans olefinic moiety, and a carboxyl group. Therefore, compound **5** was confirmed as ferulic acid having a molecular formula of $C_{10}H_{10}O_4$.

The molecular weight of compound **6** were determined as 164 by EI-MS. The characteristic absorption bands observed at 3383, 1673, 1602, 1310 and 979 $cm^{-1}$ on the IR spectrum suggested the presence of hydroxyl group, carboxyl group, aromatic ring, phenolic hydroxyl group and trans olefinic moiety, respectively. The $^1H$ NMR data indicated the signals for four aromatic protons of 1,4-disubstituted benzene and two trans olefinic protons. The $^{13}C$ NMR data of **6** were similar to those of **4** except for the chemical shift of the C-3 position. On the basis of various spectral data discussed above, this compound was identified as p-coumaric acid having a molecular formula of $C_9H_8O_3$. The chemical structures of all compounds identified above were as shown in Fig. 1. Of these, chlorogenic acid was the main compound as 38.5%.

### Antioxidative Activities of Compound 1 through 6

The antioxidative activities of all compounds identified above were investigated by measuring the hydroperoxidation of linoleic acid via radical chain reaction. Fig. 2 indicates the measured antioxidative efficiency of each of these compounds. The antioxidant activity increased in the order of p-coumaric acid<α-tocopherol <ferulic acid<caffeic acid<chlorogenic acid<4,5-dicaffeoylquinic acid<3,5-dicaffeoylquinic acid. However, this result indicated that the other phenolic acids except for p-coumaric acid were more effective than α-to-

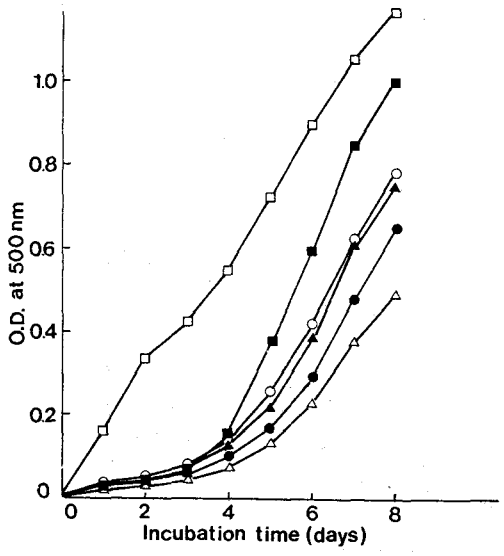


Fig. 3. Antioxidative activity of chlorogenic acid on α-tocopherol at the level of 1, 3, 5 and 10 ppm. (△—△, 10; ●—●, 5; ▲—▲, 3; ○—○, 1; □—□, control; ■—■, α-tocopherol(10 ppm)).

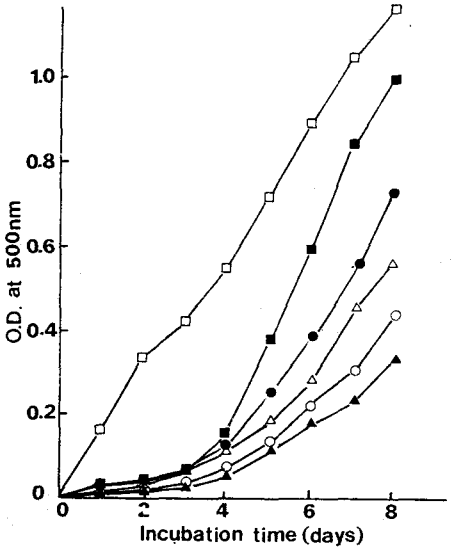


Fig. 4. Antioxidative activity of 3,5-*O*-dicaffeoylquinic acid on α-tocopherol at the level of 1, 3, 5 and 10 ppm. (▲—▲, 10; ○—○, 5; △—△, 3; ●—●, 1; □—□, control; ■—■, α-tocopherol(10 ppm)).

copherol used as a comparative standard. In other words, it may suggest that the activity of caffeoylquinic acids on a molar basis increased in proportion to the number of caffeoyl residue. On the other hand, the remaining phenolic acids except for p-coumaric and ferulic acids identified in the present study commonly possessed ortho-dihydroxyl group. Phenolic acids which have the ability to chelate metal ions by means of ortho-dihydroxyl group, may reduce the pro-oxidative activity of trace metals. In addition to observations mentioned above, phenolic acids identified from Jindalrae flowers showed a tendency to increase antioxidant effect with an increase of the number of hydroxyl group. Especially, the introduction of secondary hydroxyl group at ortho or para position is known to increase antioxidative activity.[12,13] Caffeic acid was more active than ferulic acid, indicating that methoxy substitution was less efficient than the addition of a hydroxyl group. On the other hand, a comparison of the antioxidative activity of caffeic and chlorogenic acids showed that esterification by quinic acid increased the activity of the phenolic acids in this system. Although a number of investigations related to the antioxidant activity of phenolic acids have been undertaken, their results were different depending upon the system of experiments.[14-16] Summarizing the relations between structure and antioxidant activity from the limited number of comparisons available in the present study, we concluded that the following structural characteristics were correlated with antioxidant activity: 1) Increase in the number of caffeic acid esterified with quinic acid enhanced antioxidant activity, 2) Ortho-dihydroxyl group of an aromatic ring system is essential for activity, 3) A methoxy substitution of the original ortho-dihydroxyl group decreased activity, 4) Decrease in the number of hydroxyl group in the molecular structure lowered activity. The influence of hydroxylation at the other positions in phenolic acid structure need to be investigated further.

Fig. 3 and 4 showed the evaluation of antioxidative activity of chlorogenic and 3,5-dicaffeoylquinic acids at the level of 1, 3, 5 and 10 ppm, respectively. Both phenolic acids at the level of 1 ppm have greater antioxidative activities than those of α-tocopherol at the level of 10 ppm. The antioxidative efficiency of these phenolic acids were remarkably enhanced in proportion to their concentrations. However, chlorogenic and 3,5-*O*-dicaffeoylquinic acids are the major phenolic acids so that they may play an important role in antioxidant activity of Jindalrae flowers. Since phenolic acids are widely distributed in various foods, the detailed investigations on the biological effects of the compounds would be quite valuable. Furthermore, characterization of unidentified phenolic acids is necessary and is currently in progress.

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## 진달래꽃으로부터 분리된 페놀산 화합물의 항산화성에 관한 연구

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**초록** : 우리나라에서 식용으로 이용되는 진달래꽃으로부터 6성분의 페놀산 화합물이 분리동정되었다. 이들 화합물의 구조는 IR, UV, $^{1}H$과 $^{13}C$ NMR, FAB-MS, ES-MS와 EI-MS에 의해 얻어진 분광학적인 결과에 근거하여, chlorogenic acid, 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid, caffeic acid, ferulic acid, p-coumaric acid인 것으로 밝혀졌다. Chlorogenic acid (0.2 g)는 ethyl acetate과 ethyl ether 분획에 동시에 함유되어 있었고, polyamide C-200 관 크로마토그래피법, 분취용 박층크로마토그래피법, 재결정법, Sephadex LH-20 관 크로마토그래피법을 통해서 양 구분으로부터 최종적으로 회수된 총 페놀산 함량 (0.52 g)의 38.5%를 차지하였다. 항산화성은 티오시안산철의 존재하에서 리놀레산의 에타놀 용액 중에서 측정되었다. 항산화능은 p-coumaric acid< α-tocopherol<ferulic acid<caffeic acid<chlorogenic acid<4,5-dicaffeoylquinic acid<3,5-dicaffeoylquinic acid의 순서로 증대되었다.

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