Food Sci. Biotechnol. Vol. 14, No. 1, pp. 143 ~ 146 (2005)

Food Science and Biotechnology

Flavonoid Compounds from *Viola hondoensis* and Their Effect on Matrix Metalloproteinase-1 in Ultraviolet Irradiation of Cultured Human Skin Fibroblasts

Hyung-In Moon, Joongku Lee[1], Ok Pyo Zee[2] and Jin Ho Chung***

Derpartment of Dermatology, Seoul National University College of Medicine, and Clinical research Institute, Seoul National University Hosipital, Seoul, 110-744, Korea

[1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, 305-333, Korea

[2]College of Pharmacy, Sung kyun Kwan University, Suwon, 440-746, Korea

Abstract Although many studies have been performed to elucidate the molecular consequences of ultraviolet irradiation, little is known about the effect of natural products. Ultraviolet irradiation is widely considered to be an environmental stress. Here we investigated the effect of 2',4',7-trihydroxyisoflavone on the regulation of MMP-1 and type 1 procollagen in Ultraviolet irradiation of cultured human dermal fibroblasts. Phytochemical investigation of the whole plants of *Viola hondoensis* led to the isolation of five flavonoids. The structures of these compounds were identified 2',4',5,7-tetrahydroxyisoflavone (**1**), 2',4',7-trihydroxyisoflavone (**2**), 4',7-dihydroxyflavone (**3**), isoorientin (**4**), and isovitexin (**5**) using spectroscopic analysis. Among these, 2',4',7-trihydroxyisoflavone reduced the expression of MMP-1 at the protein levels in a dose-dependent manner by ultraviolet irradiation. Taken together, our results suggest that 2',4',7-trihydroxyisoflavone an important role in the reduction of MMP-1 induction by ultraviolet irradiation.

Key words: *Viola hondoensis,* matrix metalloproteinase-1, ultraviolet irradiation, flavonoid

Introduction

Viola hondoensis W. Becker et Boissieu (Violaceae) distributed in southern part of Korea. In traditional medicine, the herb has been used as an expectorant, a diuretic, and an antiinflammatory for bronchitis, rheumatism, skin eruptions, and eczema (1,2). Previous phytochemical studies on *Viola* species have revealed them to be a rich source of cyclotides (3,4), and several flavone glycosides (5,6). This plant has not been investigated in detail, although some common triterpene saponin has been reported (7).

The matrix metalloproteinases (MMPs) are a family of >20 zinc-dependent endoproteinases that are capable of degrading almost all of the components of the extracellular matrix (8). MMPs can be divided into four categories based on substrate preference: collagenases, gelatinases, stromelysins, and membrane-associated matrix metalloproteinases (9). MMPs are necessary for tissue remodeling and the healing cascade under normal physiological condition. The aging process of skin can be divided into intrinsic aging and photoaging. Clinically, naturally aged skin is smooth, pale, and finely wrinkled. In contrast, photoaged skin is coarsely wrinkled (10). Alterations in collagen, the major structural component of skin, have been suggested as a cause of the changes, such as skin wrinkling and loss of elasticity, observed in naturally aged and photoaged skin (11). With increasing age, collagen synthesis becomes lower and MMP-1 levels become higher in sun-protected human skin *in vivo* (11). UV irradiation induces the synthesis of matrix metalloportienases (MMP) in fibroblast cell *in vitro* and MMP-mediated collagen destruction accounts, in large part, for the connective tissue damage that occurs in photoaging (10).

In an ongoing investigation into MMP-1 inhibitory compound from *V. hondoensis*, a compound was isolated from the ethylacetate(EtOAc) soluble fraction. In this paper, we report on the constituent of *V. hondoensis,* which inhibits UV-induced MMP-1 expression in primary cultured human skin fibroblasts.

Materials and Methods

General procedure IR spectra were obtained with a Perkin Elmer 1710 spectrophotometer. The NMR spectra were taken on a JEOL LA 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz, Japan), a Bruker GPX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz, Germany), and a Bruker AMX 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz, Germany) spectrometer. EIMS spectra were obtained on a VG Trio-2 spectrometer(USA). FAB-MS spectra were obtained on a JMS AX505WA spectrometer(Japan). TLC was carried out on silica gel 60 F_{254} and RP-18 F_{254} plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh, Germany) and Sephadex LH-20 (Pharmacia, Sweden).

Plant materials The whole plants of *Viola hondoensis* (Violaceae) were collected in April 2004 at Ulleung Island, Korea. The botanical identification was made by one of the authors, Dr. JoongKu Lee. A voucher specimen of this raw material deposited has been deposited at the herbarium of the Seoul National University (SNU-04-04-13).

*Corresponding author: Tel: 82-2-760-2414; Fax: 82-2-742-7344
E-mail: jhchug@snu.ac.kr
**Co-corresponding author: Tel: 82-2-760-1745; Fax: 82-2-742-7344
E-mail: himun@snu.ac.kr
Received November 30, 2004; accepted January 3, 2005

Extraction and isolation The dried whole plants (393 g) of *Viola hondoensis* were extracted five times with 80 % methanol(MeOH) in an ultrasonic apparatus for 3 hrs. This residue was evaporated *in vacuo* to yield the total extract (26.8 g). This extract was then suspended in distilled water and partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-butanol (BuOH). The EtOAc fraction (1.2 g) was subjected to silica gel column chromatography using $CHCl_3$-EtOAc gradient system (90:1 → 1:1) to provide 11 fractions (fractions 1-11). From fraction 8, compound **2** (2.2 mg) was isolated using a silica gel column chromatography ($CHCl_3$:EtOAc = 20:1), and then purified by semi-preparative RP-HPLC (YMC Jsphere-H80, 4 μm, 250 x 10 mm, MeOH:H_2O = 6:4). Compounds **1** (1.4 mg) and **3** (1.5 mg) were also separated from fraction 9 by the same procedure as those of the fraction 8. Fraction 11 was rechromatographed over silica gel and eluted with solvent mixtures ($CHCl_3$:MeOH:H_2O = 50:4:1 → $CHCl_3$:MeOH:H_2O = 8:4:1) to give six fractions (subfractions 1-6). Subfraction 6 was chromato-graphed with Sephadex LH-20 (MeOH) to provide four fractions (subfractions 7-10). Compounds **4** (4.0 mg) and **5** (5.0 mg) were further purified by RP-HPLC (Waters, Bondapak, 5 m, 300 x 3.9 mm) with solvent mixtures (AcCN:H_2O = 2:8 for **4** and MeOH:AcCN:H_2O = 7:13:80 for **5**).

2',4',5,7-tetrahydroxyisoflavone (1): Pale yellow powder; mp: 271-273°C; IR λ_{max} (KBr, cm^{-1}): 3410 (OH), 1619 (C=O), 1575, 1510, 1462 (C=C); MS (EI, *m/z*) : 286 (M^+, 100), 269 (25), 217 (8), 153 (RDA, 65), 134 (RDA, 43); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 13.03 (1H, brs, 5-OH), 8.14 (1H, s, H-2), 6.96 (1H, d, *J* = 8.3 Hz, H-6'), 6.35 (1H, d, *J* =1.6 Hz, H-8), 6.35 (1H, d, *J* = 2.3 Hz, H-3'), 6.26 (1H, dd, *J* = 8.3, 2.3 Hz, H-5'), 6.20 (1H, d, *J* = 1.6 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm) 180.8 (C-4), 164.7 (C-7), 162.3 (C-5), 159.0 (C-4'), 158.1 (C-9), 156.8 (C-2'), 155.7 (C-2), 132.6 (C-6'), 120.8 (C-3), 109.0 (C-1'), 106.6 (C-5'), 104.8 (C-10), 103.0 (C-3'), 99.3 (C-6), 94.0 (C-8)

2',4',7-trihydroxyisoflavone (2): Pale yellow powder; mp: 273-275°C; IR λ_{max} (KBr, cm^{-1}): 3410 (OH), 1619 (C=O), 1575, 1510, 1462 (C=C); MS (EI, *m/z*): 270 (M^+, 100), 269 ([M-H]$^+$, 18), 253 (38), 137 (RDA, 48), 134 (RDA, 38); ^{1}H-NMR (400 MHz, DMSO-d_6 , ppm): 8.13 (1H, s, H-2), 7.92 (1H, d, *J* = 8.8 Hz, H-5), 6.97 (1H, d, *J* = 8.3 Hz, H-6'), 6.91 (1H, dd, *J* = 8.8, 2.2 Hz, H-6), 6.84 (1H, d, *J* = 2.2 Hz, H-8), 6.34 (1H, d, *J* = 2.4 Hz, H-3'), 6.26 (1H, dd, *J* = 8.3, 2.4 Hz, H-5') ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 180.8 (C-4), 164.7 (C-7), 162.3 (C-4'), 158.1 (C-9), 156.8 (C-2'), 155.7 (C-2), 132.6 (C-6'), 120.8 (C-3), 109.0 (C-1'), 106.6 (C-5'), 104.8 (C-10), 103.0 (C-3'), 99.3 (C-6), 94.0 (C-8)

4',7-dihydroxyflavone (3): Pale yellow powder; mp: 314-316°C; MS (EI, *m/z*): 254 (M^+, 100), 226 (45), 137 (RDA, 98), 118 (RDA,52); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 7.91 (2H, d, *J* = 8.7 Hz, H-2', 6'), 7.84 (1H, d, *J* = 8.7 Hz, H-5), 6.93 (1H, s, H-8), 6.92 (2H, d, *J* =8.7 Hz, H-3', 5'), 6.89 (1H, d, *J* = 8.7 Hz, H-6), 6.71 (1H, s, H-3); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): 176.3 (C-4), 163.1 (C-7), 162.3 (C-2), 160.7 (C-4'), 157.5 (C-9), 128.1 (C-2', 6'), 126.4 (C-5), 121.7 (C-1'), 115.9 (C-10), 115.8 (C-3', 5'), 115.0 (C-6), 104.4 (C-3), 102.4 (C-8)

isoorientin (4): Light-yellow needles (MeOH/EtOAc); mp: 235-237°C; MS (FAB, *m*-NBA): 449 ([M+H]$^+$); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): 13.5 (1H, brs, 5-OH), 7.41 (1H, dd, *J* =8.3, 2.1 Hz, H-6'), 7.39 (1H, d, *J* = 2.1 Hz, H-2'), 6.88 (1H, d, *J* = 8.3 Hz, H-5'), 6.66 (1H, s, H-3), 6.47 (1H, s, H-8), 4.57 (1H, d, *J* = 9.8 Hz, H-1"), 4.07 (1H, t, *J* = 9.8 Hz, H-2"), 3.9-3.0 (H-3", H-4", H-5", H-6"); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 182. 3 (C-4), 164.1 (C-2), 163.7 (C-7), 161.1 (C-5), 156.1 (C-9), 150.1 (C-4'), 146.2 (C-3'), 121.9 (C-1'), 119.4 (C-6'), 116.5 (C-5'), 113.7 (C-2'), 109.3 (C-6), 103.8 (C-10), 103.2 (C-3), 93.9 (C-8), 82.0 (C-5"), 79.4 (C-3"), 73.5 (C-1"), 71.1 (C-2"), 70.6 (C-4"), 61.9 (C-6")

isovitexin (5): Yellow crystal (MeOH/EtOAc); mp: 237-239°C; MS (FAB, glycerol) : 433 ([M+H]$^+$); ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) : 13.5 (1H, brs, 5-OH), 7.92 (2H, d, *J* =8.8 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.77 (1H, s, H-3), 6.49 (1H, s, H-8), 4.57 (1H, d, *J* = 10.0 Hz, H-1"), 4.02 (1H, t, *J* =10.0 Hz, H-2"), 3.9-3.0 (H-3", H-4", H-5", H-6"); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 182. 4 (C-4), 163.9 (C-2), 163.8 (C-7), 161.6 (C-9), 161.1 (C-4), 156.7 (C-5), 128.9 (C-2, 6), 121.5 (C-1), 116.4 (C-3, 5), 109.3 (C-6), 103.8 (C-10), 103.2 (C-3), 94.1 (C-8), 82.0 (C-5"), 79.4 (C-1"), 73.5 (C-2"), 71.0 (C-3"), 70.6 (C-4"), 61.9 (C-6")

Primary human skin fibroblasts culture Primary cultures of skin fibroblasts were established from human adult foreskins in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum, 2 mM glutamine, and penicillin (100 U/ml), streptomycin (100 mg/ml) in a 37°C humidified incubator containing 5% CO_2. The fibroblasts were cultured until 90% confluency and then, subcultivated. Cells cultured after 5 passages were used for the experiments.

Cell proliferation assay and UV irradiation Cell proliferation was determined by the MTT assay (12), which is based on reduction of soluble yellow MTT tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to a blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase. After compound or UV treatment, the cells were cultured for indicated days; 20 ml of MTT (5 mg/ml) was added to each well, and the cells were incubated for 4 hrs at 37°C. The supernatant was removed, and 200 μl of dimethyl-sulfoxide was added to each well to dissolve formazan products. The absorbance was determined spectrophotometrically at 570 nm with an ELISA reader. The results were expressed as a percentage of control in six cultures. The UV light source was a F75/85W/UV21 fluorescent sun lamps, having an emission spectrum between 285-350 nm (peak at 310-315 nm) as previously described (7). A Kodacel filter (TA401/407; Kodak, Rochester, NY) was mounted 2 cm in front of the UV tubes to remove wavelengths <290 nm (UV-C). The fibroblasts were grown in 10 cm culture dishes (Falcon, Lincoln Park, NJ) until subconfluent. Subsequently, the cells were cultured in serum-free medium for 24 hrs, and the medium was replaced by 2 ml of phosphate-buffered saline. Then the cells were exposed to UV (0-100 mJ/cm^2) light. After irradiation, the cells were washed with phosphate-buffered saline, and cultured in the media with or without

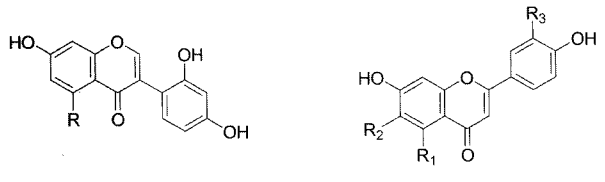
compounds for the indicated time (13).

Western blots and statistical analysis The supernatant was used for western blot analysis. A monoclonal anti-MMP-1 antibody (Oncogen, Co., Boston, MA, USA) were used as primary antibodies. Statistical significance was determined using the Student t-tests. Results are presented by means ± standard error of mean(SEM). All p values quoted are two-tailed and were accepted as significantly different when p was ≤0.05.

Results and Discussion

Phytochemical investigation of *Viola hondoensis* led to the isolation of five flavonoids from the EtOAc fraction using column chromatography and hplc. Compound **1** was a pale yellow powder, and showed characteristic phenolics in positive reaction with $FeCl_3$ reagents. The molecular formula of **1** was calculated as $C_{15}H_{10}O_6$ by EIMS and ^{13}C NMR data. The EIMS spectrum of **1** showed the molecular ion peak at *m/z* 286. Peaks from *retro*-Diels Alder fragmentation at *m/z* 153 and 134 indicated that this flavonoid has two hydroxy functionalities in its A and B rings, respectively (14). The ^1H-NMR signals showed two *meta* coupled protons [d 6.35 (1H, d, *J* =1.6 Hz, H-8), 6.20 (1H, d, *J* = 1.6 Hz, H-6)], and a hydroxy proton at 5-position [δ 13.03 (1H, brs)]. In addition, a singlet proton signal at δ 8.13 indicated a characteristic proton at the C-2 position of the isoflavonoid (15). The B-ring protons showed a characteristic 1,2,4-trisubstitued benzene ring [d 6.96 (1H, d, *J* = 8.3 Hz), δ 6.35 (1H, d, *J* = 2.3 Hz), 6.26 (1H, dd, *J*= 8.3, 2.3 Hz)] and also displayed not the 3',4'-dihydroxy but the 2',4'-dihydroxy flavonoid (15).

Therefore, the structure of **1** was suggested to be 2',4',5,7-tetrahydroxyisoflavone (2'-hydroxygenistein) and was confirmed using the ^{13}C-NMR spectrum and in comparison to previously reported data (16). The molecular formula of compound **2** was determined to be $C_{15}H_{10}O_5$ based on the ^{13}C-NMR spectral data and EIMS [*m/z* 270, M^+]. A positive reaction in $FeCl_3$ reagent also displayed the phenolic characteristic of this compound. Its ^1H-NMR spectrum and EIMS fragmentation were similar to those of compound **1**, except for presence of 1,2,4-trisubstitued benzene ring protons [δ 7.92 (1H, d, *J* = 8.8 Hz), 6.91 (1H, dd, *J*= 8.8, 2.2 Hz), 6.84 (1H, d, *J* = 2.2 Hz)] and an absence of the hydroxy group at the 5-position in A ring. In addition, the molecular ion peak at *m/z* 270 and the fragment peaks from *retro*-Diels Alder fragmentation at *m/z* 137 and 134 confirmed the one hydroxy group in its A ring and the two hydroxy groups in its B ring. Therefore, the structure of compound **2** was determined as 2',4',7-trihydroxyisoflavone. The spectral data of **2** were in good agreement with the literature values (17). The ^1H-NMR signal of compound **3** displayed *para*-substituted benzene ring [δ 7.91 (2H, d, *J* = 8.7 Hz), 6.92 (2H, d, *J* = 8.7 Hz)], disappearance of the 5-OH signal around δ 13 ppm and the existence of a 3 position proton at δ 6.71(1H, brs). In the EIMS spectrum, the molecular ion peak was shown at *m/z* 254, and the signals from *retro*-Diels Alder fragmentation at *m/z* 137 and 118 indicated the one hydorxy group in the A ring and in the B ring, respectively. These data indicated that compound **3** is 4',7-dihydroxyflavone, and this was confirmed when compared with the literature values (18). Compound **4** was obtained as light-yellow needles and showed a psedomolecular ion peak at *m/z* 449 $[M+H]^+$. The molecular formula of compound **4** was established as $C_{21}H_{20}O_{21}$ by MS and ^{13}C-NMR data. A proton signal at d 13.50 displayed the existence of OH at the 5 position and the *C*-glycosidic linkage was validated by a chemical shift of the 6 position [δ 4.57 (^1H), 109.3 (^{13}C)] and anomeric carbon signals at d 73.5 (15).The chemical shift of the A ring protons [δ 6.66 (1H, s), 6.47 (1H, s)] and the B ring protons [δ 7.41 (1H, dd, *J* = 8.3, 2.1 Hz), 7.39 (1H, d, *J* = 2.1 Hz), 6.88 (1H, d, *J* = 8.3 z)] were in accord with those of luteolin (15). ^{13}C-NMR spectrum of **4** led the identification of the sugar moiety as glucose and was in agreement with the literature values (16). In conclusion, the structure of **4** was determined to be isoorientin (19). The ^1H- and ^{13}C-NMR spectra of compound **5** were similar to those of compounds **3** and **4**. In other words, the chemical shift of **5** was almost equal to



1 R=OH

2 R=H

3 R_1=H, R_2=H, R_3=H

4 R_1=OH, R_2=Glc, R_3=OH

5 R_1=OH, R_2=Glc, R_3=H

Fig. 1. Structures of isolated compounds from *Viola hondoensis*.

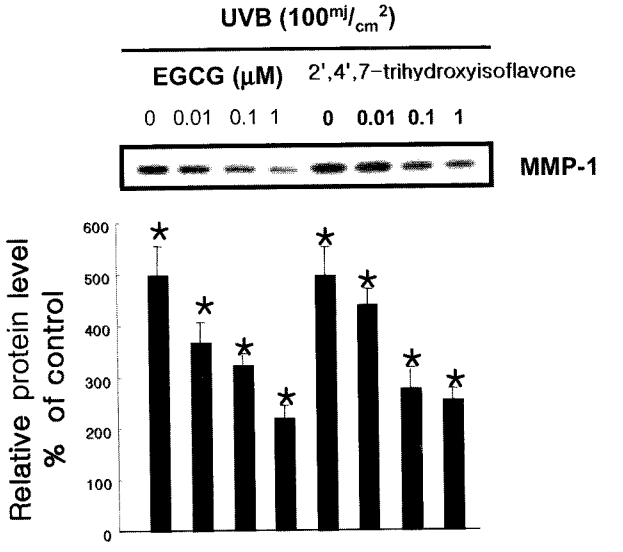


Fig. 2. 2',4',7-trihydroxyisoflavone prevent UV-induced increase in the MMP-1 expression in the culture human skin fibroblasts. The cells were pretreated with 2',4',7-trihydroxy-isoflavone prior to UV irradiation (100 mJ/cm^2) and harvested 72 hr later. Epigallo-catechin-3-gallate was used as a positive control. Each value represents a mean±SEM (n=5). *signifiantly different from control at p=0.05

those of the B ring of **3**, and the A and C rings of **4**. The ^{1}H-NMR signals displayed a *para*-substituted benzene ring B [δ 7.92 (2H, d, J = 8.8 Hz), 6.92 (2H, d, J = 8.8 Hz)], and two singlet peaks of H-3 and H-8 at δ 6.77 (1H, s) and 6.49 (1H, s), respectively. Therefore, the structure of **5** was identified as isovitexin (20). We investigated the effects of 2',4',7-trihydroxyisoflavone on the expression of MMP-1 in cultured human skin fibroblasts. The *in vitro* cytotoxicity assay was investigated according to reference (12). The compound did not show cytotoxicity against test doses (0.1-1 μM, $p<0.001$). The expression levels of MMP-1 protein were determined in culture media by western blot analysis. We have demonstrated that compared with UV - treated cells (Fig. 2), the compound decreased the UV-induced expression of MMP-1 protein in a dose-dependent manner by an average of 82.3±10.2% ($p\leq0.05$, n=5) at 0.01 μM, 70.2±1.3 % ($p\leq0.05$, n=5) at 0.1 μM, and 63.5± 8.2% ($p\leq0.05$, n=5) at 1 μM. The compound showed smilar activity to positive control, EGCG (Epigallocatechin Gallate) in the protein levels. In conclusion, 2',4',7-trihydroxyisoflavone may be used for the treatment and prevention of UV-induced expression of MMP-1 protein.

Acknowledgments

This study was supported by grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ1-PG1-CH14-0001).

References

1. Schuele S, Lederman RJ. Long-term outcome of focal dystonia in string instrumentalists. Mov Disord 19: 43-48 (2004)
2. Tennstedt D, Comphaut P, Dooms-Goossens A, Lachapelle JM. Dermatoses of the affecting violin and viola players. Derm Beruf Umwelt. 27: 165-169 (1979)
3. Lindholm P, Goransson U, Johansson S, Claeson P, Gullbo J, Larsson R, Bohlin L, Backlund A. Cyclotides: a novel type of cytotoxic agents. Mol Cancer Ther. 1: 365-369 (2002)
4. Svangard E, Goransson U, Smith D, Verma C, Backlund A, Bohlin L, Claeson P. Primary and 3-D modeled structures of two cyclotides from *Viola odorata*. Phytochemistry 64: 135-142 (2003)
5. Carnat AP, Carnet A, Fraisse D, Lamaison JL, Heitz A, Wylde R, Teulade JC. Violarvensin, a new flavone di-C-glycoside from *Viola arvensis*. J. Nat .Prod. 61: 272-274 (1998)
6. Xie C, Veitch NC, Houghtn PJ, Simmonds MS. Flavone C-glycosides from *Viola yedoensis* MAKINO. Chem. Pharm. Bull. 51: 1204-1207 (2003)
7. Moon HI, Chung JH, Lee JK, Zee OP. Triterpenoid Saponin from *Viola hondoensis* W. Becker et H Boss. and Their Effect on MMP-1 and Type I Procollagen Expression. Arch. Pharm. Res. 27:730-733 (2004)
8. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. J. Natl. Cancer Inst. 89: 1260-1270 (1997)
9. Bernhard EJ, Gruber SB, Muschel RJ. Direct evidence linking expression of matrix metalloproteinase 9 (92-kDa gelatinase/ collagenase) to the metastatic phenotype in transformed rat embryo cells. Proc. Natl. Acad. Sci. USA. 91: 42934297 (1994)
10. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S. Voorhees. J. J., Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379: 335-339 (1996)
11. Varani J, Warner R.L, Gharaee-Kermani M, Phan. SH, Kang S, Chung JH, Wang ZQ, Datta SC, Fisher GJ, Voorhees JJ. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J. Invest Dermatol. 114: 480-486 (2000)
12. Han DS, Han JH, OH SW, Rho SN. Effects of Onion Extracts on Both Normal and Neoplastic Cell Proliferation and the Inhibition of Tumor Growth in Mouse. Food Sci. Biotechnol. 11: 557-560 (2004)
13. Seo JY, Lee SH, Youn CS, Choi HR, Rhie GE, Cho KH, Kim KH, Park KC, Eun HC, Chung JH. Ultraviolet radiation increases tropoelastin mRNA expression in the epidermis of human skin in vivo. J Invest Dermatol. 116: 915-919. (2001)
14. Markham KR. Chari VM. Carbon-13 NMR spectroscopy of flavonoids, pp. 19-132 In: The Flavonoids: Advances in Research. Harborne, J.B. and Mabry, T.J. (Eds.). Chapman & Hall, London, (1982)
15. Markham KR, Geiger H. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeutero-dimethylsulfoxide. pp. 441-497 In: The Flavonoids: Advances in Research Since 1986 Harborne, J.B. (Ed.). Chapman & Hall, London, (1994)
16. Biggs R. Post-infectional compounds from the French bean *Phaseolus vulgaris*: Isolation and identification of genistein and 2',4',5,7-tetrahydroxyisoflavone. Aust. J. Chem. 28: 1389-1392 (1975)
17. Woodward MD. Phaseollin formation and metabolism in *Phaseolus vulgaris*. Phytochemistry 19: 921-927 (1985)
18. Shirataki Y, Yokoe I, Komatsu M. Two new flavone glycosides from the roots of *Sophora subprostrata*. J. Nat. Prod. 49: 645-649 (1986)
19. Kato T, Morita Y. C-glycosylflavones with acetyl substitution from *Rumex acetosa* L. Chem. Pharm. Bull. 38: 2277-2280 (1990)
20. Agrawal PK. Bansal MC. Flavonoid glycosides, pp. 283-364 In: Carbon-13 NMR of Flavonoids. Agrawal, P.K. (Ed.). Elsevier, Amsterdam, (1989)