BIOACTIVE ISOCOUMARINS FROM CISSUS PTEROCLADA

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Investigation of the roots with stems of *Cissus pteroclada* Hayata resulted in isolation of fourteen compounds including five isocoumarins, bergenin (1), norbergenin (2), 6-*O*-galloylbergenin (3), 6-*O*-(4-hydroxy benzoyl) bergenin (4), and 6-*O*-galloylnorbergenin (5). The structures of the isolated compounds were identified by spectroscopic analyses and comparison of their spectral data with those in literature. Compounds 1, 3, 5 were evaluated for anti-inflammatory activity. Both 3 and 5 showed potent inhibitory effect on superoxide anion generation with IC₅₀ values of 1.94 ± 0.33 and $4.08 \pm$ 0.49 µM, respectively.

Key words: Cissus pteroclada Hayata, Vitaceae, isocoumarin, anti-inflammatory, superoxide anion

Introduction

Cissus pteroclada Hayata (*Vitis pteroclada* Hayata, *Cissus hastate* (Miq.) Planch,^{1,2} Vitaceae) is the original plant of a traditional Chinese "Sifang-teng".³ The stems of this plant is traditionally used to treat rheumatism, cramp, contuse, and bruise.^{3,4} *C. pteroclada* has been one of our studied plants in the family Vitaceae. Previously, we isolated several resveratrol derivatives from the roots of *Vitis thunbergii* Sieb. & Zucc.,^{5,6} and they showed significant antiplatelet, antioxidative, antiinflammatory, and H1N1-stimulated RANTES secretion inhibitory activities.⁷⁻¹⁰ The plants of genus *Cissus*

have been reported to contain multiple classes of constituents. Among them, *C sicyoides* (CS) is distributed throughout the tropics, mainly in Brazil and Caribbean, it is used as a diuretic, antiinflammator, and antidiabetic. The extract of CS presented a vasoconstrictor effect on guinea-pig aorta rings and an anticonvulsant activity.¹¹ Phytochemical studies of this plant, abundant constituents including coumarins, flavonoids, anthocyanins, stilbenes, lignans, and alkaloids were isolated.¹² *C quadrangularis* (CQ) is widely distributed in India and used as an osteoprotective agent in Ayurveda, the Indian system of alternative medicine. The petroleum ether extract of this plant showed anti-osteoporotic activity

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in rats.¹³ The constituents isolated from CQ included triterpenoids, steroids, stilbenes, flavonoids, lipids, and iridoids.¹⁴ The root of *C. repens* L. is traditionally used to treat carbuncle, furuncle, cystitis, and gonorrhea in Taiwan. It was reported to contain stilbenes, lignans, and triterpenes.^{15,16} Previously, we investigated the constituents of this material, and several stilbenes were obtained.¹⁷ Earlier, Chi et al reported the isolation of six compounds from the stems of C. pteroclada, including three isocoumarins, bergenin (1), 6-O-galloylbergenin (3), and 6-O-(4-hydroxy benzoyl) bergenin (4).³ In our investigation of the constituents of this plant, five isocoumarins along with nine other compounds were obtained. Except for three isocoumarins mentioned above and gallic acid, norbergenin (2) and 6-O-galloylnorbergenin (5) with other eight compounds were isolated from this plant for the first time. Among these compounds, isocoumarins 1, 3, 5 were tested for anti-inflammatory activity.

Materials and Methods

I. General experimental procedures

Mass spectra were recorded with a Finnigan DSQ II MS spectrometer (ESI-MS). ¹H, ¹³C, and 2D NMR spectra were measured with a Varian Unity Inova 500 MHz FT-NMR spectrometer.

II. Plant material

The roots with stems of *C. pteroclada* were collected from Hsin Chu county, Taiwan in August, 2008 and identified by a taxonomist, Mr. Jun-Chih Ou. A voucher specimen (NHP-01476) is deposited at the Herbarium of National Research Institute of Chinese Medicine, Republic of China.

III. Extraction and isolation

The air-dried roots with stems of C. pteroclada (8.37 kg) were extracted at 50°C with ethanol (160 $L \times 3$, 24 h each). After evaporation of the solvent in vacuo, the concentrated EtOH extract (576.76 g) was treated with CH₂Cl₂-Me₂CO/1:1 to give CH₂Cl₂-Me₂CO/1:1-soluble (29.70 g) and CH₂Cl₂ -Me₂CO/1:1-insoluble (547.06 g) portions. The CH₂Cl₂-Me₂CO/1:1 soluble portion was subjected to a silica gel column (70-230 mesh, 10×45 cm) eluting with gradient solvent systems of CH₂Cl₂-Me₂CO (20:1 to 0:1) and MeOH to yield 65 fractions (3 L each). From fraction 4, $CH_2Cl_2-Me_2CO = 20:1$ eluate, the insoluble portion in *n*-hexane was purified by a silica gel column eluting with *n*-hexane-Me₂CO (25:1) to get taraxerol (3.2 mg) and taraxerone (5.3 mg). Fractions 21-22, $CH_2Cl_2-Me_2CO = 10:2$ eluate, was further separated over a silica gel column eluting with CH₂Cl₂-Me₂CO (20:1) to yield betulinic acid (8.3 mg). From fractions 23-29, CH₂Cl₂-Me₂CO = 10:3 eluate, the insoluble portion in CH_2Cl_2 was purified over a silica gel column eluting with CH₂Cl₂ -Me₂CO (10:1) to obtain ursolic acid (34.5 mg). Reseparation of fractions 50-53, CH₂Cl₂-Me₂CO = 10:10 eluate, through a Sephadex LH-20 column with MeOH-H₂O (3:1, 1:0) and a MPLC column [Lichroprep RP-18, 40-63 μ m, 2 × (2.5 × 31 cm); Merck] with H₂O-MeOH/90:10 to 60: 40 (v/v, flow rate: 2.0 mL/min) to give 6-O-(4-hydroxy benzoyl) bergenin (4, 3.2 mg, purity 95.9%) and methyl caffeate (2.8 mg). Fractions 54-55, CH₂Cl₂-Me₂CO = 10:15 eluate, was purified through a Sephadex LH-20 column (MeOH-H₂O, 3:1 to 1:0) to give bergenin (1, 3.38 g, purity 99.8%) and mixture A which was further purified over a MPLC column (RP-18) eluting with H₂O-MeOH/60:40 and then

through recrystallization (MeOH/H₂O) to afford 6-O-galloylbergenin (3, 96.09 mg, purity 99.2%). Using the same chromatographic methods as fractions 50-53 to treat fractions 57-58 (CH₂Cl₂-Me₂CO = 10:30 eluate), (+)-(2S,3R)-gallocatechin (4.6 mg), 1,6-di-O-galloyl-β-D-glucoside (3.1 mg), myricetrin (16.8 mg), 6-O-galloylnorbergenin (5, 138.1 mg, purity 97.0%), and norbergenin (2, 3.5 mg, purity 96.8%) were isolated. Moreover, the CH₂Cl₂-Me₂CO/1:1-insoluble portion was suspended in MeOH, and the soluble portion (445.42 g) was subjected to a Sephadex LH-20 column (MeOH-H₂O, 3:1, 5:1, 1:0) to afford 40 fractions (1 L each). Then fractions 15-18, MeOH $-H_2O = 3:1$ eluate, was repeatedly purified over MPLC columns (RP-18) eluting with H₂O-MeOH/90:10 to 60: 40 to yield gallic acid (255.6 mg) and 6-O-galloylnorbergenin (30.3 mg).

IV. Bergenin (1)

White powder; UV (CH₃OH) λ_{max} (log ε): 317 (3.44), 245 (3.91), 218 (4.42) nm; IR (KBr) y_{max} cm⁻¹: 3394 (OH), 1703 (C=O), 1616, 1465, 1346, 1231, 1093, 855; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 3.18 (1H, m, H-4'), 3.42 (1H, m, H-6'a), 3.55 (1H, t, J = 8.0)Hz, H-5'), 3.63 (1H, m, H-3'), 3.76 (3H, s, 4-OCH₃), 3.83 (1H, br d, J = 9.5 Hz, H-6'b), 3.99 (1H, m, H-2'),4.88 (1H, t, J = 5.5 Hz, 6'-OH), 4.96 (1H, d, J = 10.5 Hz, H-1'), 5.40 (1H, d, *J* = 6.0 Hz, 4'-OH), 5.61 (1H, d, J = 5.5 Hz, 3'-OH), 6.98 (1H, s, H-6), 8.43 (1H, s, 3-OH), 9.73 (1H, s, 5-OH); ¹H NMR (CD₃OD, 500 MHz) δ 3.44 (1H, t, *J* = 9.0 Hz, H-4'), 3.66 (1H, m, H-5'), 3.71 (1H, m, Hx-6'), 3.81 (1H, t, J = 9.0 Hz, H-3'), 3.88 (3H, s, 4-OCH₃), 4.01 (1H, br d, *J* = 10.5 Hz, Hy-6'), 4.06 (1H, t, J = 10.0 Hz, H-2'), 4.93 (1H, d, J = 10.5 Hz, H-1'), 7.06 (1H, s, H-6); ¹³C NMR (DMSO-*d*₆, 125 MHz) δ 59.8 (4-OCH₃), 61.1 (C-6'), 70.6 (C-4'), 72.0 (C-1'), 73.6 (C-3'), 79.7 (C-2'), 81.7 (C-5'), 109.4 (C-6), 115.9 (C-2), 118.0 (C-1), 140.5 (C-4), 148.0 (C-3), 150.9 (C-5), 163.3 (C-7); ESIMS *m/z* 327 [M–H]⁻ (C₁₄H₁₆O₉).

V. Norbergenin (2)

Brown yellow powder; UV (CH₃OH) λ_{max} (log ε): 289 (1.06), 221 (4.42) nm; IR (KBr) ν_{max} cm⁻¹: 3391 (OH), 1704 (C=O), 1622, 1479, 1348, 1234, 1091, 870; ¹H NMR (CD₃OD, 500 MHz) δ 3.43 (1H, t, *J* = 9.0 Hz, H-4'), 3.66 (1H, m, H-5'), 3.69 (1H, m, H-6'a), 3.80 (1H, t, *J* = 9.0 Hz, H-3'), 4.01 (1H, br d, *J* = 10.5 Hz, H-6'b), 4.02 (1H, t, *J* = 10.0 Hz, H-2'), 4.93 (1H, d, *J* = 10.5 Hz, H-1'), 7.06 (1H, s, H-6); ¹³C NMR (CD₃OD, 125 MHz) δ 62.7 (C-6'), 71.9 (C-4'), 74.3 (C-1'), 75.6 (C-3'), 81.3 (C-2'), 82.9 (C-5'), 111.0 (C-6), 114.2 (C-1), 117.3 (C-2), 141.2 (C-4), 143.6 (C-3), 147.2 (C-5), 166.4 (C-7); ESIMS *m/z* 313 [M– H]⁻ (C₁₃H₁₄O₉).

VI. 6-O-galloylbergenin (3)

White powder; UV (CH₃OH) λ_{max} (log ε): 276 (4.52), 219 (4.86) nm; IR (KBr) ν_{max} cm⁻¹: 3366 (OH), 1732, 1699 (C=O), 1618, , 1520, 1467, 1352, 1225, 1095, 870; ¹H NMR (CD₃OD, 500 MHz) δ 3.54 (1H, dd, J = 9.0, 10.0 Hz, H-4'), 3.84 (1H, t, J = 9.0 Hz, H-3'), 3.89 (3H, s, 4-OCH₃), 3.95 (1H, m, H-5'), 4.10 (1H, dd, J = 9.0, 10.5 Hz, H-2'), 4.38 (1H, dd, J = 7.0, 12.0 Hz, H-6'a), 4.82 (1H, br d, J = 12.0 Hz, H-6'b), 5.02 (1H, d, J = 10.5 Hz, H-1'), 7.09 (1H, s, H-6), 7.10 (2H, s, H-9, 13); ¹³C NMR (CD₃OD, 125 MHz) δ 61.0 (4-OCH₃), 64.6 (C-6'), 71.8 (C-4'), 74.4 (C-1'), 75.5 (C-3'), 80.7 (C-5'), 81.3 (C-2'), 110.3 (C-9, 13), 111.2 (C-6), 117.0 (C-1), 119.5 (C-2), 121.0 (C-8), 140.1 (C-11), 142.3 (C-4), 146.6 (C-10, 12), 149.3

(C-3), 152.4 (C-5), 165.7 (C-7), 168.2 (C-14); ESIMS *m*/*z* 479 [M–H]⁻ (C₂₁H₂₀O₁₃).

VII. 6-O-(4-hydroxy benzoyl) bergenin (4)

Brown yellow powder; UV (CH₃OH) λ_{max} $(\log \varepsilon)$: 262 (4.20), 215 (4.50) nm; IR (KBr) v_{max} cm⁻¹: 3367 (OH), 1708 (C=O), 1610, 1519, 1458, 1352, 1238, 1099, 854; ¹H NMR (CD₃OD, 500 MHz) δ 3.53 (1H, t, J = 9.0 Hz, H-4'), 3.83 (1H, t, J = 9.0 Hz H-3'),3.87 (3H, s, 4-OCH₃), 3.95 (1H, m, H-5'), 4.09 (1H, t, J = 10.0 Hz, H-2'), 4.35 (1H, dd, J = 7.0, 12.0 Hz, H-6'a), 4.89 (1H, br d, J = 12.0 Hz H-6'b), 5.00 (1H, d, *J* = 10.5 Hz, H-1'), 6.82 (2H, d, *J* = 8.5 Hz, H-10, 12) , 7.06 (1H, s, H-6), 7.91 (2H, d, *J* = 8.5 Hz, H-9, 13); ¹³C NMR (CD₃OD, 125 MHz) δ 61.0 (4-OCH₃), 64.8 (C-6'), 72.0 (C-4'), 74.3 (C-1'), 75.4 (C-3'), 80.6 (C-5'), 81.2 (C-2'), 111.2 (C-6), 116.2 (C-10, 12), 117.0 (C-2), 119.4 (C-1), 121.8 (C-8), 133.0 (C-9, 13), 142.2 (C-4), 149.3 (C-3), 152.3 (C-5), 163.7 (C-11), 165.6 (C-7), 167.9 (C-14); ESIMS m/z 447 [M-H]⁻ $(C_{21}H_{20}O_{11}).$

VIII. 6-*O*-galloylnorbergenin (5)

Brown yellow powder; UV (CH₃OH) λ_{max} (log ε): 282 (4.15), 217 (4.58) nm; IR (KBr) ν_{max} cm⁻¹: 3383 (OH), 1712 (C=O), 1622, 1536, 1450, 1352, 1234, 1087, 870; ¹H NMR (CD₃OD, 500 MHz) δ 3.52 (1H, t, *J* = 9.0 Hz, H-4'), 3.84 (1H, t, *J* = 9.0 Hz, H-3'), 3.94 (1H, m, H-5'), 4.07 (1H, t, *J* = 9.0 Hz, H-2'), 4.36 (1H, dd, *J* = 7.0, 12.0 Hz, H-6'a), 4.82 (1H, br d, *J* = 12.0 Hz, H-6'b), 5.02 (1H, d, *J* = 10.5 Hz, H-1'), 7.08 (1H, s, H-6), 7.09 (2H, s, H-9, 13); ¹³C NMR (CD₃OD, 125 MHz) δ 64.8 (C-6'), 71.9 (C-4'), 74.5 (C-1'), 75.5 (C-3'), 80.6 (C-5'), 81.3 (C-2'), 110.2 (C-9, 13), 111.1 (C-6), 114.3 (C-1), 117.0 (C-2), 121.0 (C-8), 140.1 (C-11), 141.2 (C-4), 143.5 (C-3), 146.5 (C-10, 12), 147.4 (C-5), 166.3 (C-7), 168.1 (C-14); ESIMS *m*/*z* 465 [M–H][–] (C₂₀H₁₈O₁₃).

IX. Anti-inflammatory activity

The isocoumarins **1**, **3**, **5** were assayed for antiinflammatory activity based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. The measurement of superoxide anion generation and elastase release by human neutrophils were assayed with the method described previously.^{18,19} The purities of compounds **1**, **3**, **5** determined by NMR method gave 99.8%, 99.2%, and 97.0%, respectively.

Results and Discussion

An ethanolic extract of the roots with stems of *C. pteroclada* was repeatedly chromatographed on silica gel and Sephadex LH-20 to afford fourteen compounds including five isocoumarins, bergenin (1),^{3,20} norbergenin (2),^{3,20} 6-*O*-galloylbergenin (3),^{3,21} 6-*O*-(4-hydroxy benzoyl) bergenin (4),³ and 6-*O*-galloylnorbergenin (5),²¹ along with taraxerol,²² taraxerone,²² betulinic acid,²³ ursolic acids,²³ gallic acid, (+)-(2*S*,3*R*)-gallocatechin,²⁴ 1,6-di-*O*-galloyl-β-p-glucoside,²⁵ methyl caffeate,²⁶ and myricetrin.²⁷

Compound 1 was obtained as white powder, and its molecular formula was determined to be $C_{14}H_{16}O_9$ by ESIMS ([M–H]⁻ 327) and NMR data. One aromatic methoxy and one ester carbonyl signals were observed at δ 59.8 and 163.3, respectively, in the ¹³C spectrum of 1. Other twelve signals were attributed to the carbons of one set of six-membered sugar and one aromatic ring. In the ¹H NMR spectrum , the signals at δ 3.18 (1H, m, H-4'), 3.42 (1H, m, H-6'a), 3.55 (1H, t, *J* = 8.0 Hz, H-5'), 3.63 (1H, m, H-3'), 3.83 (1H, br d, J = 9.5 Hz, H-6'b), 3.99 (1H, m, H-2'), and 4.96 (1H, d, J = 10.5 Hz, H-1') were attributed to one β -glucosyl moiety. The positions of these protons were assigned based on their ¹H-¹H COSY correlations. The remaining two singlets were due to one methoxy group (δ 3.76) and one aromatic proton (δ 6.98). In the HMBC spectrum of **1** (Fig. 1), the anomeric proton signal (δ 4.96) existed correlations with the signals of one oxygenated aromatic carbon at δ 148.0 (C-3) and two aromatic tertiary carbons at δ 118.0 (C-1) and 115.9 (C-2) which also correlated to H-2' signal, suggesting that the sugar moiety was C-C linked at C-2 of the aromatic ring through anomeric carbon. The proton signal at δ 6.98 showed correlations with the signals of C-1, C-2, and carbonyl signal (δ 163.3), which resulted in assignment of this proton to H-6. In addition, the signals of two hydroxys at δ 8.43 (3-OH) and 9.73 (5-OH) existed correlations with the signals of C-2 and C-6 (δ 109.4), respectively, and another carbon (δ 140.5) that was thus assigned to C-4 which also correlated to the methoxy protons. Therefore, this methoxy group was judged to be at C-4. Since the proton signals of other three hydroxys were observed at δ 4.88 (6'-OH), 5.40 (4'-OH), and 5.61 (3'-OH) which were assigned based on their ¹H-¹H COSY correlations, and the signal of H-2' appeared at lower field than those of other sugar protons except H-1'. This suggested the linkage of C-2'



Fig. 1. HMBC correlations of 1 and 3.

to C-1 through an ester group, which was supported by the molecular weight of **1**. According to the above spectral analyses, and comparison of these data with those in literature,^{3,20} compound **1** was identified as bergenin.

Compound **2** was obtained as brown yellow powder and had a molecular formula of $C_{13}H_{14}O_9$ ([M -H]⁻ 313) that differed by a CH₂ moiety from **1**. Its ¹H and ¹³C NMR spectra as well as 2D NMR crrelations were closely similar to those of **1**, except for the absence of one methoxy signal. Comparison of the spectral data of **2** with those in literature,^{3,20} this compound was identified as norbergenin.

Compound **3**, $C_{21}H_{20}O_{13}$ [M–H]⁻ 479, was obtained as white powder. The spectral characteristics of compound **3** showed it was a derivative of bergenin (**1**). The ¹³C and DEPT NMR spectra displayed the presence of nineteen signals for twenty-one carbons including one aromatic methoxy (δ 61.0) and two ester carbonyl carbons (δ 165.7, 168.2). In the ¹H NMR spectrum of **3**, in addition to the signals of bergenin portion, one symmetric AA'-type singlet at δ 7.10 (H-9, 13) were displayed. This signal existed HMBC correlations (Fig. 1) with the signals of its HMQC correlated carbon at δ 110.3 (C-9, 13), three oxygenated carbons at δ 140.1 (C-11) and 146.6 (C-10, 12), and the carbonyl carbon at δ 168.2 (C-14), which indicated the presence of one galloyl substitute that was suggested to link at C-6' of the glucosyl moiety based on the correlation between H₂-6' (δ 4.38, 4.82) and C-14. According to the above evidence and comparison of the spectral data of **3** with those in literature,^{3,21} this compound was identified as 6-*O*-galloylbergenin.

Compound 4, brown yellow powder, was also a derivative of bergenin and had a molecular formula of $C_{21}H_{20}O_{11}$ ([M–H]⁻ 447) that was two oxygen less than that of **3**. In the ¹H NMR spectrum of 4, the signals due to a set of A_2X_2 type aromatic protons appeared at δ 6.82 (2H, d, J = 8.5 Hz, H-10, 12) and 7.91 (2H, d, J = 8.5 Hz, H-9, 13) which existed HMBC correlation (Fig. 1) with the carbonyl carbon at δ 167.9 (C-14). In addition, correlations between H₂-6' (δ 4.35, 4.89) and C-14 were also displayed. Accordingly, compound 4 was identified as 6-O-(4-hydroxy benzoyl) bergenin.³ Compound 5 was obtained as brown yellow powder and had a molecular formula of $C_{20}H_{18}O_{13}$ ([M–H]⁻ 465) that differed by a CH₂ moiety from **3**. Its ¹H and ¹³C NMR spectra as well as 2D NMR crrelations were closely similar to those of 3, except for the absence of one methoxy signal. Compound 5 was thus identified as

 Table 1. Effects of compounds on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.

Compounds	Superoxide anion	Elastase release	
	$IC_{50} (\mu M)^{a} \text{ or } (Inh \%)^{b}$	IC ₅₀ (μM) or (Inh %)	_
bergenin (1)	(12.48 ± 5.95)	(25.47 ± 8.63)	*
6-O-galloylbergenin (3)	1.94 ± 0.33	(46.21 ± 4.84)	**
6-O-galloynorlbergenin (5)	4.08 ± 0.49	(8.43 ± 7.87)	

^a IC_{50} represents the 50% inhibitory concentration of the compound.

^b If 50% inhibition was not reached at any test dose, the percentage of inhibition obtained at a test dose of 30 μ M is given in parentheses (Inh%).

Results are presented as mean \pm S.E.M. (n = 3-4). *p <0.05, **p <0.001 compared with the control value.

6-O-galloylnorbergenin.²¹

With the exception of **2** and **4**, which were insufficient in quantity for additional analysis, other three isocoumarins **1**, **3**, **5** were tested for antiinflammatory activity based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. The results were shown in Table 1, 6-*O*-galloylbergenin (**3**) exhibited the most potent effect in the superoxide anion assay with an IC₅₀ value of $1.94 \pm 0.33 \mu$ M. Obviously, both **3** and **5**, bearing an galloyl moiety at C-6' position, were more active than **1**. All three compounds showed no significant effect against elastase release.

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References

- Editorial Committee of the Flora of Taiwan, Second Edition. Flora of Taiwan (2nd ed.). Department of Botany, National Taiwan University, Taipei, Vol. 3, p. 702, 1993.
- Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita. Flora Reipublicae Popularis Sinicae. Science Press: Beijing, Vol. 48, p. 62, 1998.
- Chi CY, Wang F, Lei T, Xu SY, Hong AH, Cen YZ. Studies on the chemical constituents from *Cissus pteroclada. Zhong Yao Cai*, 33:1566-1568,

2010.

- Zhonghua Bencao Editorial Committee, Chinese State Administration of TCM. Shanghai Scientific and Technical Press, Shanghai, Vol. 5, pp. 287-288, 1999.
- Huang YL, Tsai WJ, Shen CC, Chen CC. Resveratrol derivatives from the roots of *Vitis thunbergii. J. Nat. Prod.*, 68:217-220, 2005.
- Chiou WF, Shen CC, Chen CC, Lin CH, Huang YL. Oligostilbenes from the roots of *Vitis thunbergii. Planta Med.*, 75:856-859, 2009.
- Ku KT, Huang YL, Huang YJ, Chiou WF. Miyabenol A inhibits LPS-induced NO production via IKK/IkB inactivation in RAW 264.7 macrophages: possible involvement of the p38 and P13K pathways. J. Agric. Food Chem., 56: 8911-8918, 2008.
- Chiou WF, Ku KT, Huang YL. Miyabenol A exhibits anti-inflammatory activity through down-regulation of LPS-induced NF-κB and JAK/STAT pathways in RAW 264.7 macrophages. J. Chin. Med., 19:73-82, 2008.
- Huang YL, Loke SH, Hsu CC, Chiou WF. (+)-Vitisin A inhibits influenza A virus-induced RANTES production in A549 alveolar epithelial cells through interference with Akt and STAT₁ phosphorylation. *Planta Med.*, 74:156-162, 2008.
- Ong ET, Hwang TL, Huang YL, Lin CF, Wu WB. Vitisin B, a resveratrol tetramer, inhibits migration through inhibition of PDGF signaling and enhancement of cell adhesiveness in cultured vascular smooth muscle cells. *Toxicol. Appl. Pharmacol.*, 256:198-208, 2011.
- de Almeida ER, de Oliveira Rafael KR, Couto GBL, Ishigami ABM. Anxiolytic and anticonvulsant effects on mice of flavonoids, linalool,

and α-tocopherol presents in the extract of leaves of *Cissus sicyoides* L. (Vitaceae). *J. Biomed. Biotechnol.*, 2009:1-6, 2009.

- 12. Xu F, Matsuda H, Hata H, Sugawara K, Nakamura S, Masayuki Y. Structures of new flavonoids and benzofuran-type stilbene and degranulation inhibitors of rat basophilic leukemia cells from the Brazilian herbal medicine *Cissus sicyoides*. *Chem. Pharm. Bull.*, 57:1089-1095, 2009.
- Potu BK, Rao MS, Nampurath GK, Chamallamudi MR, Nayak SR, Thomas H. Anti-osteoporotic activity of the petroleum ether extract of *Cissus quadrangularis* Linn. in ovariectomized Wistar rats. *Chang Gung Med. J.*, 33:252-257, 2010.
- Singh G, Rawat P, Maurya R. Constituents of Cissus quadrangularis. Nat. Prod. Res., 21: 522-528, 2007.
- Lin QF. Studies on the chemical constituents of *Cephalotaxus wilsoniana* Hayata and *Cissus repens* Lam. M.S. Thesis, Chinese Culture University, Taipei, 1996.
- Wang YH, Zhang ZK, He HP, Wang JS, Zhou H, Ding M, Hao XJ. Stilbene C-glucosides from *Cissus repens. J. Asian Nat. Prod. Res.*, 9: 631-636, 2007. (abstract)
- Chen-Yu S. A study on the constituents of the roots of *Cissus repens*. M.S. Thesis, National Taitung University, Taitung, 2009.
- Chang HL, Chang FR, Chen JS, Wang HP, Wu YH, Wang CC, Wu YC, Hwang TL. Inhibitory effects of 16-hydroxycleroda-3,13(14)*E*-dien-15-oic acid on superoxide anion and elastase release in human neutrophils through multiple mechanisms. *Eur. J. Pharmacol.*, 586:332-339, 2008.
- 19. Hwang TL, Leu YL, Kao SH, Tang MC, Chang

HL. Viscolin, a new chalcone from *Viscum coloratum*, inhibits human neutrophil superoxide anion and elastase release via a cAMP-dependent pathway. *Free Rad. Biol. Med.*, 41:1433-1441, 2006.

- Taneyama M, Yoshida S, Kobayashi M, Hasegawa H. Isolation of norbergenin from Saxifraga stolonifera. Phytochemistry, 22:1053-1054, 1983.
- Saijo R, Nonaka G, Nishioka I. Gallic acid esters of bergenin and norbergenin from *Mallotus japonicus*. *Phytochemistry*, 29:267-270, 1990.
- 22. Chen KS, Chang FR, Chia YC, Wu TS, Wu YC. Chemical constituents of *Neolitsea parvigemma* and *Neolitsea konishii*. J. Chin. Chem. Soc., 45: 103-110, 1998.
- Chang CW, Wang JP, Wu PP, Kuo SC, Lee PD. Terpenoids from Ocimum basilicum. Chin. Pharm. J., 51:181-189, 1999.
- Cai Y, Evans FJ, Roberts MF, Phillipson JD, Zenk MH, Gleba YY. Polyphenolic compounds from *Croton lechleri. Phytochemistry*, 30:2033-2040, 1991.
- 25. Nonaka G, Nishioka I. Tannins and related compounds. X. Rhubarb (2): Isolation and structures of a glycerol gallate, gallic acid glucoside gallates, galloylglucoses and isolindleyin. *Chem. Pharm. Bull.*, 31:1652-1658, 1983.
- 26. Canonica L, Danieli B, Ferrari G, Krepinsky J. A novel method of isolation of phytoecdysones from Kaladana seeds. *Phytochemistry*, 14: 525-527, 1975.
- 27. Aritomi M, Kawasaki T. Three highly oxygenated flavone glucuronides in leaves of *Spinacia oleracea*. *Phytochemistry*, 23:2043-2047, 1984.

翼莖粉藤之ISOCOUMARIN類 抗發炎活性成分研究

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翼莖粉藤 (*Cissus pteroclada* Hayata) 屬於葡萄科 (Vitaceae) 粉藤屬植物,又名四方藤、 翼莖山葡萄。其粗莖具舒筋活絡、去瘀生新之效,用於治風濕疼痛、四肢攣急、跌打內傷等。 粉藤屬植物的化學成分被報導含 coumarin、flavonoid、steronoid、triterpenoid 及 stilbene 等多類 化合物。本植物的根莖部藥材經乙醇抽取濃縮後,經由管柱層析分離、純化得到十四個化合 物,包括五個 isocoumarin類化合物—bergenin (1)、norbergenin (2)、6-*O*-galloylbergenin (3)、 6-*O*-(4-hydroxy benzoyl) bergenin (4)與6-*O*-galloylnorbergenin (5)。除了化合物1、3、4與 gallic acid外,其他十個化合物均首次分離自本植物。所得各成分的構造係經質譜及核磁共振等光譜 資料的分析,並與文獻資料比對鑑定之。對化合物1、3、5進行抗發炎活性試驗,結果化合物 3、5對嗜中性白血球所引起的超氧自由基生成有良好的抑制效果,其IC₅₀分別為1.94±0.33與 4.08 ± 0.49 µM。

關鍵字:翼莖粉藤、葡萄科、isocoumarin、抗發炎、超氧自由基