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Original Article

Hydrolysable Tannins from *Cercidiphyllum japonicum* Bark[1]

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ABSTRACT

The EtOAc and H_2O soluble fractions of Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) bark extracts were chromatographed on a Sephadex LH-20 column with various aqueous MeOH. Gallic acid (**1**), methyl galate (**2**), kurigalin (**3**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**4**) and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**5**) were isolated from EtOAc fraction. Isocorilagin (**6**) and methyl galate (**2**) were separated from H_2O fraction. The structure determination was done by ^{1}H and ^{13}C NMR. Of these isolated compounds, methyl galate (**2**), kurigalin (**3**) and isocorilagin (**6**) were isolated, for the first time, from the bark extracts of *Cercidiphyllum japonicum*.

Keywords : Katsura tree (*Cercidiphyllum japonicum*) bark, Hydrolysable tannins, gallotannin, ellagitannin, column chromatography

1. INTRODUCTION

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc), is the only species belonging to Cercidiphyllum genus, which is well represented in the fossil record, with occurrences in the late Cretaceous and Tertiary of North America and Europe. However, it is now confined to East Asian countries (Manchester *et al*., 2009). The tree is a long-lived, deciduous, wind-pollinated tree with dimorphic leaves and up to 30 to 45 m tall with a symmetrical canopy and new growth is reddish turning a light pale green. Fall color is a spectacular yellow, with some red. Thus, it is valued as an ornamental or a shade tree for landscape (Zhang *et al*., 2009). It is also a commercially and ecologically valuable one and likely to become one of the medicinal tree species. The clustered pod-like fruits contain numerous small seeds which adapted for wind dispersal. The natural populations of the tree inhabit distribute sites (600 to 2000 m) of temperate deciduous forests scattered across East China and Japan (Isagi *et*

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al., 2005).

Plants constitute a rich source of bioactive chemicals (Kador *et al*., 1985a, 1985b; Williamson *et al*., 1992). Since many plants are largely free from adverse effects and have excellent pharmacological actions, they could possibly lead to the development of new classes of safer functional agents and hydrolyzable tannins are one of those sources.

Hydrolyzable tannins are mixtures of poly-galloyl glucoses and/or poly-galloyl quinic acid derivatives containing in between 3 up to 12 gallic acid residues per molecule.

Gallotannins are polymers when gallic acid, a polyphenol monomer, esterifies and binds formed with the hydroxyl group of a polyol carbohydrate such as glucose (Cammann *et al*., 1989; Niehaus and Gross, 1997; Niemetz and Gross, 2001).

Ellagitannins are a diverse class of hydrolyzable tannins, a type of polyphenol primarily formed from the oxidative linkage of galloyl groups in 1,2,3,4,6-pentagalloyl glucose (Kwon and Bae, 2009; Sepulveda *et al*., 2011; Steinmetz, 2010).

Ellagitannins contain various numbers of hexahydroxydiphenoyl (HHDP) units, as well as galloyl units and/or sanguisorboyl units bounded to sugar moiety (Yoshida *et al*., 2009).

Recently, there have been many studies to evaluate biological activities of various natural resources, including plants and tree species and to develop pharmaceutical or functional food or cosmetic products.

However, there are little studies on katsura tree extracts for developing functional and pharmaceutical products abroad (Tada and Sakurai, 1991; Takasugi and Katui, 1986; Towatari *et al*., 2002; Nonaka *et al*., 1989).

Thus, this work was carried out to investigate the chemical constituents of the extracts of katsura tree bark for future functional and pharmaceutical use of the species.

2. MATERIALS and METHODS

2.1. Plant material

Fresh *Cercidiphyllum japonicum* barks were collected at Samcheok, Gangwon-do in June 2014, air dried for two weeks and then ground to fine particles to be extracted with Wiley mill.

2.2. Sample preparation

The ground barks (1.55 kg) were immersed in 70 % aqueous acetone at room temperature for 3 days. After three times extraction and filtration, the filtrates were combined together and evaporated on a vacuum evaporator under the reduced pressure at 40℃. The aqueous crude residue was successively fractionated on a separatory funnel and then freeze dried to give *n*-hexane (1.77 g), dichloromethane (1.48 g), ethylacetate (31.11 g), and H_2O (56.33 g) soluble fractions.

2.3. Structure analysis

^{1}H and ^{13}C NMR (Nuclear magnetic reso-

nance) spectra were recorded on a Bruker Avance DPX 300, 400 and 700 MHz spectrometers using TMS (Tetramethylsilane) as an internal standard and chemical shift was given in δ (ppm).

FAB-MS (Fast atom bombardment mass spectrometry) and MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) were performed with a Micromass Autospec M363 spectrometer.

TLC (Thin Layer Chromatography) was done on DC-Plastikfolien Cellulose F (Merck) plates and developed with TBAW (tertiary butanol-acetic acid-H_2O (3 : 1 : 1, v/v/v)) and 6% aqueous acetic acid. The spot was detected by illuminating ultraviolet light (UV, 254 and 365 nm).

2.4. Column chromatography

A portion of the EtOAc fraction (5 g) was chromatographed on a Sephadex LH-20 column by successively eluting with MeOH-H_2O (1 : 9 → 3 : 7 → 1 : 1 → 2 : 1 → 3 : 1, v/v) to afford 12 fractions, and finally acetone-H_2O (1 : 1, v/v) to give fraction 13.

Compound **1** and compound **3** were isolated from fraction 7 and 8, respectively. Fraction 12 was retreated with MeOH-H_2O (3 : 1, v/v) to isolate the compound **2**, **4** and **5**.

A portion of the H_2O fraction (6 g) was also successively treated with MeOH-H_2O (1 : 3 → 1 : 2 → 1 : 1 → 2 : 1 → 3 : 1, v/v) to afford 14 fractions, and finally acetone-H_2O (1 : 1, v/v) to get fraction 15. Fraction 6 was retreated with MeOH to isolate the compound **2** and **6**.

2.4.1. Compound **1**

Light yellow powder. R_f : 0.75 (TBAW) and 0.31 (6% HOAc).

EI-MS: Calculated for $C_7H_6O_5$ 170, Found *m/z* 170 $[M]^+$.

^1H NMR (CD_3OD, δ): 7.13 (2H, *s*, H-2, 6).

^{13}C NMR (CD_3OD, δ): 109.94 (C-2, 6), 122.43 (C-1), 138.60 (C-4), 145.83 (C-3, 5), 170.78 (C-7).

2.4.2. Compound **2**

Brownish amorphous powder. R_f : 0.76 (TBAW) and 0.34 (6% HOAc).

EI-MS: Calculated for $C_8H_8O_5$ 184, Found *m/z* 184 $[M]^+$.

^1H NMR (400 MHz, δ, CD_3OD): 3.82 (3H, s, methoxyl H), 7.05 (2H, s, H-2, 6).

^{13}C NMR (CD_3OD, δ): 52.32 (methoxyl C), 110.06 (C-2, 6), 121.47 (C-1), 139.79 (C-4), 146.43 (C-3, 5), 169.06 (C-7).

2.4.3. Compound **3**

Yellow amorphous powder. R_f : 0.19 (6% HOAc).

FAB-MS: Calculated for $C_{27}H_{24}O_{18}$ 636, Found *m/z* 635 $[M-H]^-$.

^1H NMR (CD_3OD, δ): See Table 1.

^{13}C NMR (CD_3OD, δ): See Table 2.

2.4.4. Compound **4**

Yellow amorphous powder. R_f : 0.16 (TBAW), 0.19 (6% HOAc).

FAB-MS: Calculated for $C_{34}H_{28}O_{22}$ 788, Found *m/z* 787 $[M-H]^-$.

Table 1. ^{1}H NMR chemical shifts of the isolated compounds.

Position	^{1}H NMR chemical shift				
	3(α)	3(β)	4	5	6
Galloyl					
H-2 and H-6	7.08 (A^{α}), 7.09 (A^{β}), 7.11 (B), 7.12 (C) s		6.93 (B) s 7.03 (A) s 7.04 (C) s 7.12 (D) s	6.91 (C) s 6.96 (B) s 6.99 (D) s 7.06 (A) s 7.12 (E) s	7.04 (A) s
Sugar					
H-1	5.24 s	5.32 s	6.09 d	6.22 d	6.36 d
H-2			5.45 dd	5.55 dd	3.98 d
H-2'	4.26, 4.29 brs	4.42 brs			
H-3	3.88 d	4.15 d	5.56 dd	5.89 t	4.79 brs
H-4	4.25 ddd	4.20 ddd	3.9-4.0 m	5.61 t	4.45 d
H-5	4.31, 4.51 dd	4.31, 4.51 dd	3.9-4.0 m	4.43 m	4.52 t
H-6			4.55, 4.6 dd	4.40, 4.52 dd	4.15 dd 4.95 t
HHDP					
H-6					6.66 (B) s
H-6'					6.68 (C) s

^{1}H NMR (CD_3OD, δ): See Table 1.

^{13}C NMR (CD_3OD, δ): See Table 2.

2.4.5. Compound **5**

Yellow amorphous powder. R_f: 0.2 (6% HOAc).

FAB-MS: Calculated for $C_{41}H_{32}O_{26}$ 940, Found *m/z* 939 $[M-H]^-$.

^{1}H NMR (CD_3OD, δ): See Table 1.

^{13}C NMR (CD_3OD, δ): See Table 2.

2.4.6. Compound **6**

Brownish amorphous powder. R_f : 0.25 (TBAW), 0.31 (6% HOAc).

MALDI-TOF-MS : Calculated for $C_{27}H_{22}O_{18}$ 634, Found *m/z* 657 $[M+Na]^+$.

^{1}H NMR (CD_3OD, δ): See Table 1.

^{13}C NMR (CD_3OD, δ): See Table 2.

3. RESULTS and DISCUSSION

The compounds were isolated from the EtOAc and H_2O fraction of the extracts of *Cercidiphyllum japonicum* bark by column chromatography using Sephadex LH-20, and the structures were characterized by NMR analysis and by comparison with the other literature data.

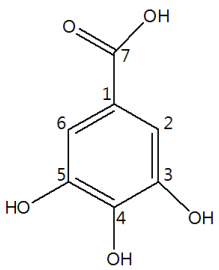
3.1. Compound **1**

Compound **1** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (1 : 1, v/v) eluent. Molecular formula of $C_7H_6O_5$ was supported by a molecular ion peak at *m/z* 170 $[M]^+$ in the EI-MS spectrum. ^{1}H NMR spectrum showed one singlet at δ 7.13 (2H, s,

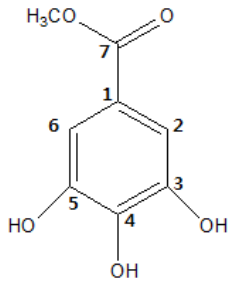
Table 2. ^{13}C NMR chemical shifts of the isolated compounds.

Position	^{13}C NMR chemical shift				
	3(α)	3(β)	4	5	6
Galloyl					
C-1	121.00 (A^{α}), 121.17 (A^{β}), 121.35 (B), 121.47 (C)		120.30 (A) 120.85 (B) 121.42 (C) 121.67 (D)	119.86 (A) 120.35 (D) 120.37 (B) 120.49 (C) 121.18 (E)	119.61 (A)
C-2 and C-6	110.15, 110.24§		110.47 (D) 110.66 (B) 110.75 (C) 110.83 (A)	110.48 (E) 110.52 (C) 110.52 (B) 110.61 (D) 110.77 (A)	109.94 (A)
C-3 and C-5	146.47, 146.54§		146.69 (B) 146.75 (C) 146.83 (A) 146.94 (D)	146.30 (C) 146.40 (B) 146.46 (D) 146.48 (E) 146.57 (A)	145.38 (A)
C-4	139.91, 140.0§		140.37 (D) 140.40 (B) 140.61 (C) 140.67 (A)	140.03 (E) 140.16 (C) 140.33 (B) 140.38 (D) 140.79 (A)	139.40 (A)
C-7	168.17 (A^{α}), 168.24 (A^{β}), 168.40 (B), 168.70 (C)		166.74 (A) 167.61 (B) 168.15 (C) 168.60 (D)	166.28 (A) 166.98 (D) 167.06 (B) 167.35 (C) 167.98 (E)	165.69 (A)
Sugar					
C-1	98.96	102.89	94.34	93.9	94.02
C-2	77.59	80.78	72.79	72.3	68.48
C-2'	66.49	67.55			
C-3	77.32	74.21	76.90	74.2	70.66
C-4	80.19	81.45	70.07	69.9	64.02
C-5	65.64	67.09	77.06	74.5	75.20
C-6			64.42	63.2	61.47
HHDP					
C-1					124.45 (B)
C-2					107.32 (B)
C-3					144.21 (B)
C-4					137.18 (B)
C-5					144.31 (B)
C-6					116.20 (B)
C-7					167.52 (B)
C-1'					124.49 (C)
C-2'					109.15 (C)
C-3'					144.63 (C)
C-4'					136.68 (C)
C-5'					145.04 (C)
C-6'					115.70 (C)
C-7'					169.11 (C)

§ not assigned.



Compound **1**



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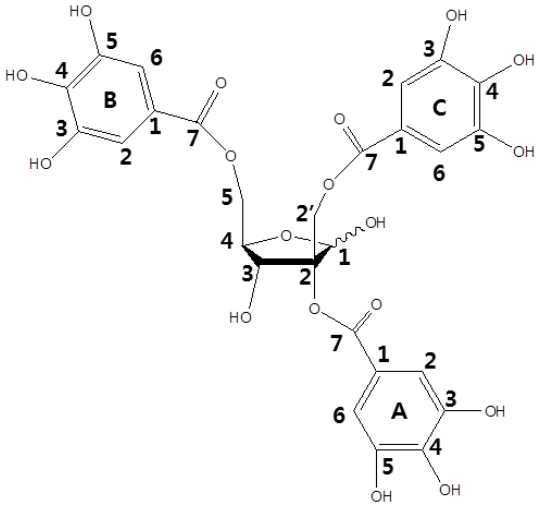

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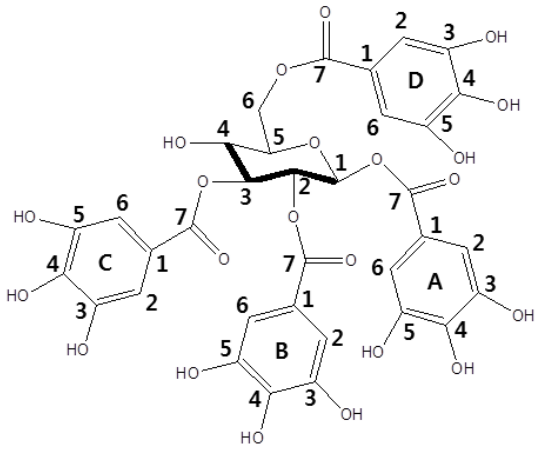
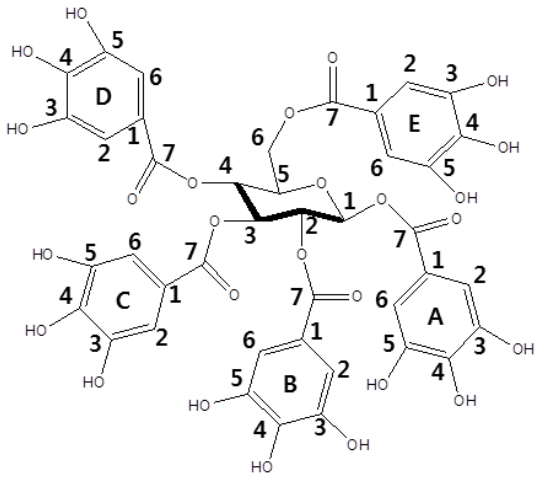
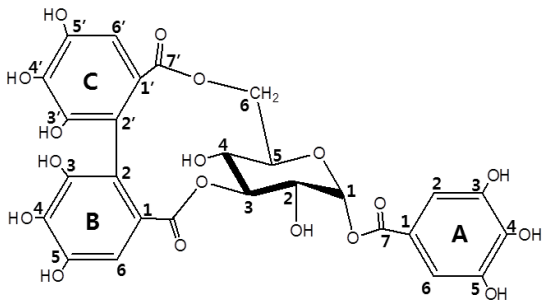


Compound **4**



Compound **5**



Compound **6**

H-2,6) from a pair of symmetric galloyl protons. ^{13}C NMR spectrum exhibited seven carbon signals, including one carbonyl carbon at 170.78 ppm (C-7) and two pairs of symmetric galloyl carbons at 109.94 (C-2,6) and 145.83 ppm (C-3,5). Therefore, the structure of compound **1** was identified as gallic acid (3,4,5-trihydroxybenzoic acid) (Luo *et al*., 2009; Kashiwada *et al*., 1988; Kwon, 2010; Saijo *et al*., 1990).

3.2. Compound 2

Compound **2** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (3 : 1, v/v) eluent. The molecular formula of $C_8H_8O_5$ was established on the basis of a peak at *m/z* 184 $[M]^+$ in the EI-MS spectrum. ^1H NMR spectrum showed a pair of symmetric galloyl protons at δ 7.05 (H-2,6) and one methoxyl proton signal at δ 3.82. ^{13}C NMR spectrum exhibited a carbonyl carbon at 169.06 ppm, two pairs of symmetric galloyl carbons at 110.06 (C-2,6) and 146.43 ppm (C-3,5), and one methoxyl carbon at 52.32 ppm. Therefore, the structure of compound **2** was elucidated as methyl gallate (3,4,5-trihydroxybenzoic acid methyl ester) (Lee and Jeong, 2005; Kwon, 2010).

3.3. Compound 3

Compound **3** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (1 : 1, v/v) eluent. The molecular formula was determined to be $C_{27}H_{24}O_{18}$ by FAB-MS (*m/z* 635 $[M-H]^-$).

In the ^1H NMR spectrum, the two anomeric proton signals of H-1 of the sugar indicated at δ 5.24 for α-anomer and δ 5.32 for β-anomer, respectively. H-3 gave two doublets at δ 3.88 and δ 4.15 for α- and β-anomer, respectively. H-4 also gave two multiple signals for α- and β-anomer at δ 4.25 and δ 4.20, respectively. H-5 showed two pairs of double doublets at δ 4.31 and δ 4.51 for α-anomer and β-anomer. Two methylene protons of H-2' gave a broad singlet at δ 4.26 and δ 4.29 for α-anomer and δ 4.42 for β-anomer. These proton signals were very similar to those of hamamelos (Lampire *et al*., 1998; Ozawa *et al*., 1984; Wang *et al*., 2012).

Two pairs of symmetrical H-2 and H-6 of the galloyl groups (A and C), which is attached to C-2 of hamamelose, showed at δ 7.08 and δ 7.09 for A, and δ 7.12 for C. Another pair of symmetrical H-2 and H-6 of the galloyl (B) bound to C-4 gave a singlet at δ 7.11. These proton signals were also identical to the literature data (Lampire *et al*., 1998; Ozawa *et al*., 1984; Wang *et al*., 2012).

In the ^{13}C NMR chemical shifts of α-hamamelose, C-1 gave a signal at 98.96 ppm and hydroxyl containing C-3 appeared at 77.32 ppm. Galloyl A ring containing C-2 showed a signal at 77.59 ppm and galloyl C ring containing C-2' indicated a signal at 66.49 ppm. Methine carbon C-4 gave a signal at 80.19 ppm and C-5 containing galloyl B ring was at 65.64 ppm. β-hamamelose gave signals at 102.89 ppm for C-1, 80.78 ppm for C-2, and 67.55 ppm for

C-2'. Also C-3, C-4 and C-5 showed signals at 74.21, 81.45 and 67.09 ppm, respectively. These ^{13}C NMR values were also identical to the literature (Wang *et al*., 2012).

Galloyl A ring C-1 gave two signals at 121.0 ppm and 121.17 ppm for α- and β-anomer, respectively. Galloyl B and C rings also showed signals at 121.35 and 121.47 ppm for C-1, respectively. Carbonyl C-7 of A ring appeared at 168.17 and 168.24 ppm for α- and β-anomer, respectively. Carbonyl C-7 of B and C rings also indicated at 168.40 and 168.70 ppm, respectively. One symmetrical carbons, C-2 and C-6, gave two signals at 110.15 and 110.24 ppm for three galloyl rings and could not be assigned. Also another symmetrical carbons, C-3 and C-5, also appeared at 146.47 and 146.54 ppm for the galloyl rings and did not assigned too. Hydroxyl containing C-4 gave signals at 139.91 and 140.0 ppm without assignment.

This ^{13}C NMR was also close to the previous literature data (Lampire *et al*., 1998; Ozawa *et al*., 1984; Wang *et al*., 2012) and compound 3 was identified as kurigalin (2,2',5-tri-*O*-galloyl-α,β-D-hamamelose).

3.4. Compound **4**

Compound **4** was isolated from Sephadex LH-20 colum chromatography using MeOH-H_2O (3 : 1, v/v) solvent. Molecular formula of $C_{34}H_{28}O_{22}$ was established on the basis of the FAB-MS (*m/z* 787 $[M-H]^-$).

^1H NMR spectrum of the sugar moiety gave a signal of galloyl A containing H-1 at δ 6.09 with 8.2*Hz* of coupling constant indicating β-D-glucose. Hydroxyl containing H-4 and methine H-5 showed multiple signals at δ 3.9-4.0 together. Also H-2 and H-3 having galloyl B and C indicated double doublet signals at δ 5.46 and δ 5.56, respectively. Galloyl D ring containing H-6 appeared at δ 4.55 and δ 4.60 for two methylene protons. These proton signals were very similar to those of the previous literature values (Duan *et al*., 2004; Owen *et al*., 2003).

Four sets of symmetrical protons (H-2 and H-6) of four galloyl rings showed signals at δ 6.93 (B), δ 7.03 (A), δ 7.04 (C), and δ 7.12 (D), respectively.

^{13}C NMR of the sugar indicated six signals at 94.34 ppm (C-1), 72.79 ppm (C-2), 76.90 ppm (C-3), 70.07 ppm (C-4), 77.06 ppm (C-5) and 64.42 ppm (C-6), respectively.

In the galloyl rings, A ring carbon signals were resonated at 120.30 ppm (C-1), 140.67 ppm (C-4) and 166.74 ppm (carbonyl C-7), respectively. Two sets symmetrical carbons (C-2 and C-6, C-3 and C-5) gave signals at 110.83 and 146.83 ppm, respectively. The other galloyl rings, B, C and D, also indicated similar carbon chemical shifts to those of A ring.

Based on ^1H and ^{13}C NMR data and the previous literature values (Duan *et al*., 2004; Owen *et al*., 2003), compound **4** was characterized as 1,2,3,6-tetra-*O*-galloyl-β-D-glucose. (Duan *et al*., 2004; Owen *et al*., 2003).

3.5. Compound **5**

Compound **5** was isolated from Sephadex LH-20 colum chromatography using MeOH-H_2O (3 : 1, v/v) eluting solvent. Molecular formula was determined to be $C_{41}H_{32}O_{26}$ by FAB-MS (*m/z* 939 $[M-H]^-$).

^1H and the ^{13}C NMR spectra of compound **5** were also very similar to those of compound **4**.

^1H NMR spectrum of the sugar showed a signal of galloyl A containing H-1 at δ 6.22 with 8.3*Hz* of coupling constant indicating β-D-glucose. H-4 containing galloyl D ring was downfield shifted about δ 5.61 compare to compound **4** due to the conjugation effect of galloyl group. The other proton chemical shifts of the glucose were very close to those of compound **4**. Also five pairs of symmetrical protons (H-2 and H-6) of the galloyl rings gave five signals at δ 6.91 (C), δ 6.96 (B), δ 6.99 (D), δ 7.06 (A), and δ 7.12 (E). These were almost same as the literature data (Lee *et al*. 2015; Sancheti *et al*., 2011; Tanaka *et al*., 1985; Wang *et al*., 2012).

In the ^{13}C NMR spectrum, D-glucose gave six signals at 93.9 ppm (C-1), 72.3 ppm (C-2), 74.2 ppm (C-3), 69.9 ppm (C-4), 74.5 ppm (C-5) and 63.2 ppm (C-6). Also five galloyl rings indicated similar carbon chemical shifts each other. C-1 was 119.86 ppm to 121.18 ppm, C-4 was 140.03 ppm to 140.79 ppm and carbonyl C-7 was 166.28 ppm to 167.98 ppm. Two pairs of symmetrical carbons appeared at 110.48 ppm to 110.77 ppm for C-2 and C-6, and at 146.30 ppm to 146.57 ppm for C-3 and C-5.

These carbon chemical shifts were very close to the literature (Lee *et al*. 2015; Sancheti *et al*., 2011; Tanaka *et al*., 1985; Wang *et al*., 2012).

Based on the above spectral data and the literature data, compound **5** was elucidated as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose.

3.6. Compound **6**

Compound **6** was isolated by Sephadex LH-20 colum chromatography using MeOH-H_2O (1 : 3, v/v) followed by MeOH solvent.

The positive MALDI-TOF-MS spectrum showed a sodium complex $[M+Na]^+$ at *m/z* 657, corresponding to the molecular formula $C_{27}H_{22}O_{18}$.

^1H NMR spectrum indicated one galloyl ring and one HHDP unit connected to D-glucose. A galloyl containing H-1 of the sugar indicated a signal at δ 6.36 (coupling constant 1.69*Hz*) suggesting α-anomeric glucose. H-3 and H-6 connecting to HHDP unit gave signals at δ 4.79 for H-3, and at δ 4.15 and δ4.95 for H-6. The other hydroxyl containing H-2 and H-4 appeared at δ 3.98 and δ 4.45, respectively. H-5 also gave a triplet signal at δ 4.52. These proton signals were very identical to those of α-anomer of glucose. A symmetrical protons of galloyl A ring showed a signal at δ 7.04 for H-2 and H-6. Also H-6 of B ring and H-6' of C ring consisting of HHDP unit indicated two singlet signals at δ 6.66 and δ 6.68, respectively.

These proton signals were very identical to those of the previous authentic data (Chung *et al*., 2003; Kwon, 2010; Liu *et al*., 2008).

The ^{13}C NMR spectrum showed three carbonyl signals at 165.69 ppm (galloyl, C-7), 167.52 ppm (HHDP, C-7(B)), 169.11 ppm (HHDP, C-7'(C)). The oxygenated aromatic carbons of the substituted and free galloyl moieties were observed between 136.68 ppm and 145.04 ppm. Methine aromatic carbon signals were present at 109.94 (galloyl, C-2 and C-6), 116.20 (HHDP, C-6) and 115.70 (HHDP, C-6'), and quaternary carbon signals at 119.61 (galloyl, C-1), 124.45 (HHDP, C-1), 107.32 (HHDP, C-2), 124.49 (HHDP, C-1'), and 109.15 (HHDP, C-2'). These results indicated the presence of one galloyl and one HHDP moiety in the molecule (Latté *et al*., 2000). In the glucose moiety, an anomeric carbon signal was observed at 94.02 ppm (C-1). C-2 (68.48 ppm), C-3 (70.66 ppm) and C-6 (61.47 ppm) signals were downshifted 1∼4 ppm, compare to those of the original α-glucose (Anderson *et al*., 2006), indicating that the galloyl groups were attached to C-2, C-3 and C-6 of the α-glucose. These carbon chemical shifts were very similar to those of the literature (Chung *et al*., 2003; Liu *et al*., 2008).

On the basis of the above spectral data, compound **6** was identified as isocorilagin (1-*O*-galloyl-3,6-(R)-hexahydroxy-diphenoyl (HHDP)-α-D-glucopyranose) (Chung *et al*., 2003; Kwon, 2010; Liu *et al*., 2008).

4. CONCLUSION

A portion of the EtOAc fraction (5 g) was chromatographed on a Sephadex LH-20 column by successively eluting with MeOH-H_2O (1 : 9 → 3 : 7 → 1 : 1 → 2 : 1 → 3 : 1, v/v) to afford 12 fractions, and finally acetone-H_2O (1 : 1, v/v) to give fraction 13.

Compound **1** and compound **3** were isolated from fraction 7 and 8, respectively. Fraction 12 was retreated with MeOH-H_2O (3 : 1, v/v) to isolate the compound **2**, **4** and **5**.

A portion of the H_2O fraction (6 g) was also successively treated with MeOH-H_2O (1 : 3 → 1 : 2 → 1 : 1 → 2 : 1 → 3 : 1, v/v) to afford 14 fractions, and finally acetone-H_2O (1:1, v/v) to get fraction 15. Fraction 6 was retreated with MeOH to isolate compound **2** and **6**.

The isolated compounds were elucidated as gallic acid (**1**), methyl galate (**2**), kurigalin (**3**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**4**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**5**), Isocorilagin (**6**) using spectral and the previous literature data.

Some of these compounds were reported, for the first time, from the extracts of *C. japonicum* barks. Especially, methyl galate (**2**), kurigalin (**3**) and isocorilagin (**6**) were reported, for the first time, in the barks of *C. japonicum*. And these compounds can be used a valuable index marker for the tree species.

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REFERENCES

Anderson, O.M., Markham, K.R. 2006. Flavonoids: Chemistry, Biochemistry and Applications. Taylor & Francis, New York.

Cammann, J., Denzel, K., Schilling, G., Gross, G.G. 1989. Biosynthesis of gallotannins: β-Glucogallin-dependent formation of 1,2,3,4,6-pentagalloylglucose by enzymatic galloylation of 1,2,3,4-tetragalloylglucose. Archives of Biochemistry and Biophysics 273: 58～63.

Chung, S.K., Nam, J.A., Jeon, S.Y., Kim, S.I., Lee, H.J., Chung, T.H., Song, K.S. 2003. A prolyl endopeptidase-inhibiting antioxidant from *Phyllanthus ussurensis*. Archives of Pharmacal Research 26(12): 1024～1028.

Duan, D., Li, Z., Luo, H., Zhang, W., Chen, L., Xu, X. 2004. Antiviral compounds from traditional Chinese medicines *Galla Chinese* as inhibitors of HCV NS3 protease. Bioorg Med Chem Lett 14: 6041～6044.

Isagi, Y., Kudo, M., Osumi, K., Sato, K., Sakio, H. 2005. Polymorphic microsatellite and markers for a relictual angiosperm *Cercidiphyllum japonicum* Sieb. et Zucc. and their utility for *Cercidiphyllum magnificum*. Mol. Ecol. Notes 5: 596～598.

Kador, P.F., Robison, W.G., Kinoshita, J.H. 1985a. The pharmacology of aldose reductase inhibitors. Annu. Rev. Pharmacol. Toxicol. 25: 691～714.

Kador, P.J., Konishita, J.H., Sharpless, N.E. 1985b. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. J. Med. Chem. 28: 841～849.

Kashiwada, Y., Nonaka, G., Nishioka, I. 1988. Galloylsucroses from *Rhubarbs*. Phytochemistry 27: 1469～1472.

Kwon, D.J. 2010. Chemical constituents and biological activities of certain *Acer* Species growing in Korea. Ph.D. Dissertation, 211～221.

Kwon, D.J., Bae, Y.S. 2009. Ellagitannins from bark of *Juglans mandshurica*. Mokchae Konghak. 37(5): 480～485.

Lampire, O., Mila, I., Raminosoa, M., Michon, V., Herve, C., Penhoat, D., Faucheur, N., Laprevote, O., Scalbert, A. 1998. Polyphenols isolated from the bark of *Castanea sativa* Mill. Chemical structures and auto-association. Phytochemistry 49: 623～631.

Latté, K.P., Kolodziej, H. 2000. Pelargoniins, new ellagitannins from *Pelargonium reniforme*. Phytochemistry 54(7): 701～708.

Lee, H.Y., Jeong, H.S. 2005. Isolation and identification of antimicrobial substance from *Canavalia gladiata*. Food Science and Biotechnology 14(2): 268～274.

Lee, T.S., Bae, Y.S. 2015. A Gallotannin from *Cercidiphyllum japonicum* Leaves. J. Korean Wood Sci. Technol. 43(5): 558～565.

Liu, X., Cui, C., Zhao, M., Wang, J., Luo, W., Yang, B., Jiang, Y. 2008. Identification of phenolics in the fruit of emblica and their antioxidant activities. Food Chemistry 109(4): 909～915.

Luo, W., Zhao, M., Yang, B., Shen, G., Rao, G. 2009. Identification of bioactive compounds in *Phyllenthus emblica* L. fruit and their free radical scavenging activities. Food chemistry 114(2): 499～504.

Manchester, S.R., Chen, Z.D., Lu, A.M., Uemura, K. 2009. Eastern asian endemic seed plant genera and their paleogeographic history throughout the northern hemisphere. J. Syst. Evol. 47: 1～42.

Niehaus, J.U., Gross, G.G. 1997. A gallotannin degrading esterase from laves of Pedunculate oak.

Phytochemistry 45(8): 1555～1560.

Niemetz, R., Gross, G.G. 2001. Gallotannin biosynthesis: β-glucogallin: hexagalloyl 3-*O*-galloyltransferase from *Rhus typhina* leaves. Phytochemistry 58: 657～661.

Nonaka, G., Ishimatsu, M., Ageta, M., Nishioka I. 1989. Tannins and related compounds. LXXVI. Isolation and Characterization of Cercidinins A and B and Cuspinin, Unusual 2,3-(R)-Hexahydroxydiphenoyl Glucoses from *Cercidiphyllum japonicum* and *Castanopsis Cspidata* var. *sieboldii*. Chem. Pharm. Bull. 37(1): 50～53.

Ozawa, T., Kobayashi, S., Seki, R., Imagawa, H. 1984. A new gallotannin from bark of chestnut tree, *Castanea crenata* Sieb. et Zucc. Agric Biol Chem 48: 1411～1416.

Owen, R.W., Haubner, R., Hull, W.E., Erben, G., Spiegelhalder, B., Bartsch, H., Haber, B. 2003. Isolation and structure elucidantion of the major individual polyphenols in carob fibre. Food and Chemical Toxicology 41: 1727～1738.

Saijo, R., Nonaka, G.I., Nishioka, I. 1990. Gallica acid esters of bergenin and norbergenin from *Mallotus Japonicus*. Phytochemistry 29: 267～270.

Sancheti, S., Sancheti, S., Bafna, M., Seo, S.Y. 2011. 2,4,6-Trihydroxybenzaldehyde as a potent antidiabetic agent alleviates postprandial hyperglycemia in normal and diabetic rats. Medicinal Chemistry Research. 20: 1181～1187.

Sepulveda, L., Ascacio, A., Raul, R.H., Antonio, A.C., Cristobal, N.A. 2011. Ellagic acid: Biological properties and Biotechnological development for production processes. African Journal of Biotechnology. 10(20): 4518～4523.

Steinmetz, W.E. 2010 NMR assignment and characterization of proton exchange of the ellagitannin granatin B. Magnetic Resonance in Chemistry. 48: 565～570.

Tada, M., Sakurai, K. 1991. Antimicrobial compound from *Cercidiphyllum japonicum*. Phytochemistry 30(4): 1119～1120.

Takasugi, M., Katui, N. 1986. A biphenyl phytoalexin from *Cercidiphyllum japonicum*. Phytochemistry 25(12): 2751～2752.

Tanaka, T., Nonaka, G., Nishioka, I. 1985. Punicafolin, an ellagitannin from the leaves of *Punica grantum*. Phytochemistry 24: 2075～2078.

Towatari, K., Yoshida, K., Mori, N., Shimizu, K.,Kondo, R., Sakai, K. 2002. Polyphenols from the heartwood of *Cercidiphyllum japonicum* and their effects on prolifefation of mouse hair epithelial cells. Planta Medica 68: 995～998.

Wang, D., Kasuga, J., Kuwabara, C., Endoh, K., Fukushi, Y., Fujikawa, S., Arakawa, K. 2012. Presence of supercooling-facilitating hydrolyzable tannins in deep supercooling xylem parenchyma cells in *Cercidiphyllum japonicum*. Planta 235: 747～759.

Williamson, J., Kilo, C., Tilton, R.G. 1992. Mechanism of glucose and diabetes-induced vascular dysfunction. *In* N. Ruderman, J. Brownlee and J. Williamson (eds.), hyperglycemia, diabetes, and vascular disease. American physiology society. New York, pp. 107～132.

Yoshida, T., Hatano, T., Ito, H., Okuda, T., Quideau, S. 2009. Structural diversity and an timicrobial activities of ellagitannins. Chemistry and Biology of Ellagitannins. 55～93, World Scientific Publishing, Singapore.

Zhang, X.Y., Yuan, X.Y., Ma, J., Yuan, L.J. 2009. Research on tissue culture and regeneration of *Ceridiphyllum japonicum*. Northern horticulture (9): 77～79 (in Chinese).