J. Korean Wood Sci. Technol. 2016, 44(4): 551~558 pISSN: 1017-0715 eISSN: 2233-7180
http://dx.doi.org/DOI:10.5658/WOOD.2016.44.4.551

Original Article

# A Polyoxygenated Ellagitannin from *Cercidiphyllum japonicum* Bark[1]

Min-Sung Lee[2] · Hee-Jeong Min[2] · Jin-Kyu Kim[3] · Young-Soo Bae[2,†]

**ABSTRACT**

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) bark was collected, air-dried and extracted with 70% aqueous acetone, then concentrated and sequentially fractionated using *n*-hexane, methylene chloride ($CH_2Cl_2$), ethylacetate (EtOAc), and $H_2O$. The $H_2O$ fraction was chromatographed on a Sephadex LH-20 column with various aqueous MeOH eluting solvents to isolate an ellagitannin. The isolate was elucidated as macabarterin, a polyoxygenated ellagitannin by NMR analysis, including HSQC, HMBC, Q-TOF MS, and with the comparison of authentic literature data. The compound was an ellagitannin which was isolated, for the first time, from the extracts of Katsura tree bark, and has never been reported before in domestic tree or plant sources.

***Keywords :*** *Cercidiphyllum japonicum* bark, $H_2O$ fraction, macabarterin, 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). 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 al*., 2005).

A hydrolyzable tannin or pyrogallol-type tannin is a type of tannin that, on heating with hydrochloric or sulfuric acids, yields gallic or ellagic acids, and they 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 (Haslam, 1989). Ellagitannins are a diverse class of hy-

[1] Date Received June 1, 2016, Date Accepted July 7, 2016
[2] Department of Forest Biomaterials Engineering, College of Forest and Environment Sciences, Kangwon National University, Chuncheon 24341, Korea
[3] Bio-Center, Gyeonggi Institute of Science & Technology Promotion, Suwon 16229, Korea
[†] Corresponding author: Young-Soo Bae (e-mail: bae@kangwon.ac.kr)

drolyzable 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. In order to determine the quantity of every individual unit, the hydrolysis of the extracts with trifluoroacetic acid in methanol/water system is performed. Hexahydroxydiphenic acid, created after hydrolysis, spontaneously lactonized to ellagic acid, and sanguisorbic acid to sanguisorbic acid dilactone, while gallic acid remains intact (Yoshida *et al*., 2009).

Recently there have been many studies to evaluate biological activities of various natural resources and to develop pharmaceutical or functional food or cosmetic products. However, there are little studies on katsura tree extracts for functional uses in domestic or abroad (Tada and Sakurai, 1991; Takasugi and Katui, 1986; Towatari *et al*., 2002). This study was carried out to investigate the extracts of katsura tree bark, and to elucidate the structure of a polyoxygenated ellagitannin isolated from the extracts.

## 2. MATERIALS and METHODS

### 2.1. Plant material

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

### 2.2. Sample preparation

The ground bark (1.6 kg) was 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 reduced pressure at 40 ℃. The residue was successively fractionated on a separatory funnel and freeze dried to give *n*-hexane (1.77 g), $CH_2Cl_2$ (1.48 g), EtOAc (31.1 g), and $H_2O$ (56.3 g) soluble fractions.

### 2.3. ESI-Q-TOF MS

The following ESI-Q-Tof (AB SCIEX Triple Tof 5600+, Miami, UK) conditions, quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer, were used: ionspray voltage, 5.5 kV; declustering potential (DP), 80 V; the turbo spray temperature, 500℃; nebulizer gas (Gas 1) of 50 psi; heater gas (Gas 2), 50 psi; curtain gas, 25 psi. Nitrogen was kept as nebulizer and auxiliary gas. The TOF MS scan was operated with the mass range of m/z 300-1800. Recalibration was carried by EasyMass Accuracy® device before analysis.

### 2.4. Structure analysis

$^{1}H$ and $^{13}C$ NMR spectra, including 2D-NMR such as HSQC (Heteronuclear Single Quantum Coherence), HMBC (Heteronuclear Multiple

Bond Correlation), were recorded on a Bruker (USA) Avance DPX 700 MHz spectrometers using TMS (Tetramethylsilane) as an internal standard and chemical shift was given in $\delta$ (ppm). Thin layer chromatography (TLC) was done on DC-Plastikfolien Cellulose F (Merck) plates and developed with TBAW (*t*-BuOH-HOAc-$H_2O$ (3 : 1 : 1, v/v/v)) and 6% aqueous HOAc. The spot was detected by illuminating ultraviolet light (UV, 254 and 365 nm).

## 2.5. Column chromatography

A portion of $H_2O$ fraction (6.0 g) was chromatographed on a Sephadex LH-20 column, eluting with MeOH-$H_2O$ (1 : 3, v/v) to afford 8 fractions. Fraction 8 was retreated with MeOH-$H_2O$ (3 : 1, v/v) to isolate the polyoxygenated ellagitannin (Fig. 1).

Yellowish amorphous powder

$R_f$ : 0.02 (TBAW) and 0.40 (6% HOAc).

Q-TOF-MS : Found *m/z* 1103 $[M+H]^+$.

$^1H$ (700 MHz) and $^{13}C$ (700 MHz) NMR : See Table 1.

# 3. RESULTS and DISCUSSION

The compound was isolated from the $H_2O$ fraction of the extracts of katsura tree (*Cerdidiphyllum japonicum* Sieb, Et Zucc) bark by column chromatography using Sephadex LH-20, and the structure was elucidated by NMR analysis and comparison with the literature data.

In the $^1H$ NMR spectrum, D-(+)-glucose of

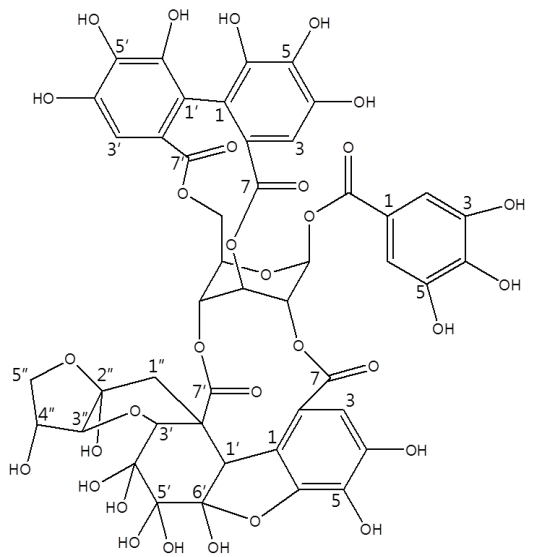

**Fig. 1.** Structure of the isolated compound.

the compound showed a double doublet signal at $\delta$ 4.37 and $\delta$ 4.82 for two H-6 protons and H-5 gave a triplet signal at $\delta$ 4.80. H-2 and H-4 indicated a doublet signal at $\delta$ 5.49 and $\delta$ 5.25, respectively. H-3 also showed a singlet signal at $\delta$ 5.54. H-1 gave a doublet signal at $\delta$ 6.51 with 8.37 *Hz* of the coupling constant suggesting the $\beta$ -anomeric glucose. These proton signals were very close to the previous literature data (Ngoumfo *et al*., 2008). The one galloyl symmetrical protons, which is attached to C-1 of D-(+)-glucose, indicated a singlet signal at $\delta$ 7.08 for H-1 and H-6 (Kwon, 2010; Xianbin *et al*., 2009). Also two HHDP (hexahydroxydiphenoyl) galloyl protons, which are bound to C-3 and C-6 of D-(+)-glucose, indicated two singlets at $\delta$ 6.87 and $\delta$ 6.63 for H-3 and H-3', respectively (Ngoumfo *et al*., 2008; Xianbin *et al*., 2009). Above proton sig-

**Table 1.** $^{1}H$, $^{13}C$, and HMBC data of the isolated compound ($CD_3OD$)

| Compound | Chemical Shift ($\delta$) | | | assignment |
|---|---|---|---|---|
| | $^{1}H$-NMR | $^{13}C$-NMR | HMBC | |
| Glucose | 6.51 brs | 92.44 | 2,3,5,7 (Galloyl) | 1 |
| | 5.49 d | 70.79 | 1,3,4,7 (Acyl group) | 2 |
| | 5.54 brs | 63.53 | 1,2,4,5,7 (HHDP | 3 |
| | 5.25 d | 66.65 | 2,3 | 4 |
| | 4.80 m | 73.97 | 1,4 | 5 |
| | 4.37 dd, 4.82 m | 64.72 | 4,5,7' (HHDP) | 6 |
| Galloyl | 7.08 s | 120.15 | | 1 |
| | | 110.91 | 1,3,4,6,7 | 2 |
| | | 146.61 | | 3 |
| | | 140.89 | | 4 |
| | | 146.61 | | 5 |
| | 7.08 s | 110.91 | 1,2,4,5,7 | 6 |
| | | 166.25 | | 7 |
| HHDP | 6.87 s | 117.59 | | 1 |
| | | 124.50 | | 2 |
| | | 110.44 | 1,2,4,5,7 | 3 |
| | | 145.41 | | 4 |
| | | 138.71 | | 5 |
| | | 145.61 | | 6 |
| | | 167.52 | | 7 |
| | 6.63 s | 116.31 | | 1' |
| | | 125.54 | | 2' |
| | | 108.24 | 1',2',4',5',7' | 3' |
| | | 146.16 | | 4' |
| | | 137.64 | | 5' |
| | | 145.66 | | 6' |
| | | 170.10 | | 7' |
| Acyl group | 7.32 s | 111.95 | | 1 |
| | | 119.72 | | 2 |
| | | 115.34 | 1,2,4,5,7 | 3 |
| | | 146.53 | | 4 |
| | | 139.34 | | 5 |
| | | 145.36 | | 6 |
| | | 166.64 | | 7 |
| | 4.78 s | 52.57 | 1,2,6,3",5',6',7' | 1' |
| | | 54.04 | | 2' |
| | 4.99 s | 77.72 | 1",4',3",1',7' | 3' |
| | | 99.42 | | 4' |
| | | 99.52 | | 5' |
| | | 99.04 | | 6' |
| | | 171.41 | | 7' |
| | 1.56 d, 2.70 d | 32.77 | 1',2',3',3",7' | 1" |
| | | 110.19 | | 2" |
| | 4.17 brs | 82.17 | 4",5" | 3" |
| | 4.09 m | 78.00 | 2",3",5" | 4" |
| | 3.93 dd, 4.23 dd | 75.65 | 2",3",4" | 5" |

nals were very similar to those of corilagin and isocorilagin reported by the literatures (Kwon, 2010; Ngoumfo *et al*., 2008; Xianbin *et al*., 2009). The acyl group gave very wide range of proton signals. One galloyl proton attached to the C-2 of D-(+)-glucose gave a singlet at $\delta$ 7.32 for H-3. Also H-1' and H-3' of the acyl group attached to the C-4 of D-(+)-glucose gave two singlets at $\delta$ 4.78 and $\delta$ 4.99, respectively. H-1" of another acyl ring indicated two doublet signals at $\delta$ 1.56 and $\delta$ 2.70 for two methylene protons. H-3" proton gave a broad singlet at $\delta$ 4.17 and one H-4" proton showed a multiplet signal at $\delta$ 4.09. Two H-5" hydroxy methylene protons gave two double doublet signals at $\delta$ 3.93 and $\delta$ 4.23.

This $^{1}$H NMR spectrum was similar to the previous literature data reported by the literature (Khallouki *et al*., 2007; Ngoumfo *et al*., 2008; Sakanaka *et al*., 1989).

In the $^{13}$C NMR spectrum, C-6, C-5 and C-4 of D-glucose gave three signals at 64.72, 73.97, and 66.65 ppm, respectively. C-1 of D-glucose indicated at 92.44 ppm, and C-2 and C-3 also showed signals at 70.79 and 63.53 ppm, respectively. These carbon signals were similar to those of the authentic sample (Ngoumfo *et al*., 2008). The galloyl carbons attached to C-1 of D-glucose gave six signals at 120.15 and 140.89 ppm for C-1 and C-4, respectively. Also two pairs of symmetrical carbons appeared at 110.91 ppm for C-2 and C-6, and 146.61 ppm for C-3 and C-5. The carbonyl C-7 gave a signal at 166.25 ppm (Kwon, 2010; Ngoumfo *et al*., 2008; Xianbin *et al*., 2009). HHDP carbons attached to C-3 and C-6 of D-glucose indicated also 14 signals. The galloyl moiety of glucose C-3 showed three signals at 110.44, 117.59 and 124.50 ppm for C-3, C-1 and C-2, respectively. C-5, C-4 and C-6 of the galloyl appeared at 138.71, 145.41 and 145.61 ppm, respectively, and the carbonyl C-7 gave a signal at 167.52 ppm (Kwon, 2010; Ngoumfo *et al*., 2008; Xianbin *et al*., 2009). The galloyl moiety of glucose C-6 also gave signals at 108.24, 116.31 and 125.54 ppm for C-3', C-1' and C-2', respectively. The other signals for C-5', C-6' and C-4' appeared at 137.64, 145.66 and 146.16 ppm, respectively. The carbonyl C-7' showed a signal at 170.10 ppm (Kwon, 2010; Ngoumfo *et al*., 2008; Xianbin *et al*., 2009).

In the acyl group, the galloyl moiety attached to the C-2 of D-glucose gave three signals at 111.95, 115.34 and 119.72 ppm for C-1, C-3 and C-2, respectively. Also the other carbons of the moiety were resonated at 139.34, 145.36 and 146.53 ppm for C-5, C-6 and C-4, respectively. The carbonyl of the moiety appeared at 166.64 ppm. In the other carbonyl containing cyclohexyl ring attached to C-4 of D-glucose, three signals for C-1', C-2' and C-3' were resonated at 52.57, 54.04 and 77.72 ppm, respectively. Also the hydroxyl containing C-6', C-4' and C-5' of the ring showed at 99.04, 99.42 and 99.52 ppm, respectively. The other heterocyclic pyran and furan ring carbons appeared at 32.77 and 75.65 ppm for C-1" and C-5", respectively. Also C-4", C-3" and C-2" gave signals at 78.0, 82.17 and 110.19 ppm, respectively. These carbon signals were very

similar to those of the authentic sample isolated by Ngoumfo *et al*..

In the HSQC spectrum, $\delta$ 4.37 (H-6) of D-glucose was correlated with 64.72 ppm (C-6). Also $\delta$ 4.80 (H-5) and 73.97 ppm (C-5), 5.25 (H-4) and 66.65 ppm (C-4), 5.54 (H-3) and 63.53 ppm (C-3), $\delta$ 5.49 (H-2) and 70.79 ppm (C-2), and 6.51 (H-1) and 92.44 ppm (C-1) were correlated each other, respectively. In the galloyl ring bound to C-1 of D-glucose, $\delta$ 7.08 (H-2 and H-6) was resonated with 110.91 ppm of C-2 and C-6. Also in the HHDP structure, H-3 ($\delta$ 6.87) of the galloyl attached to C-3 (110.44 ppm) of D-glucose was correlated and another H-3' ($\delta$ 6.63) of the galloyl attached to C-6 of D-glucose was also correlated with C-3' (108.24 ppm). In the acyl group, H-3 of the galloyl ring bound to C-2 of D-glucose had a correlation with C-3 of the ring at 115.34 ppm. The HSQC spectra of the acyl group indicated correlations between H-1' ($\delta$ 4.78) and C-1' (52.57 ppm), H-3' ($\delta$ 4.99) and C-3' (77.72 ppm), H-1" ($\delta$ 1.56) and C-1" (32.77 ppm), H-3" ($\delta$ 4.17) and C-3" (82.17 ppm), H-4" ($\delta$ 4.09) and C-4" (78.0 ppm), and H-5" ($\delta$ 3.93) and C-5" (75.65 ppm). These HSQC correlation data were very identical to the literature by Ngoumfo *et al*..

HMBC spectral data of the compound were also shown in Table 1 and the correlations with heteroatom of the molecule were also indicated in Fig. 2. H-1 of D-glucose was resonated with the carbonyl C-7 of the galloyl group. Also H-2 of D-glucose was correlated with the carbonyl C-7 of the galloyl of the acyl group. The other carbonyl C-7' of the acyl group was resonated with H-4 of D-glucose. H-3 and H-6 of D-glucose had correlations with the carbonyl C-7 and the other carbonyl C-7' of HHDP moiety, respectively. These HMBC correlations were also identical to the literature (Ngoumfo *et al*., 2008).

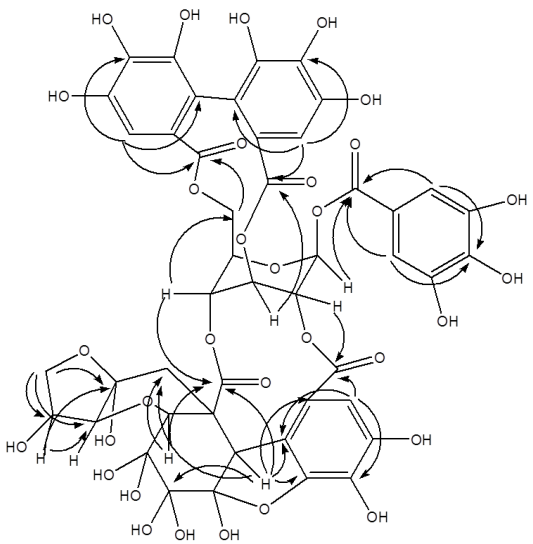

**Fig. 2.** Selected HMBC correlations observed between heteroatoms of the compound.

Based on the above results by $^{1}H$ and $^{13}C$ NMR, HSQC, HMBC, and ESI-Q-Tof MS, this compound was characterized as macabarterin and its spectral data was identical to the authentic compound (Ngoumfo *et al*. 2008). This type of polyoxygenated ellagitannin was isolated, for the first time, from katsura tree bark and has never been reported in domestic plant sources. Also we consider that this compound can be an important valuable index marker to distinct the species.

## 4. CONCLUSION

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) bark were collected, air-dried and extracted with 70% aqueous acetone. The extracts were concentrated and then sequentially fractionated with *n*-hexane, $CH_2Cl_2$, EtOAc, and $H_2O$ to be freeze dried. A portion of $H_2O$ fraction was chromatographed on a Sephadex LH-20 column, eluting with MeOH-$H_2O$ (1 : 3 and 3 : 1, v/v) to isolate the polyoxygenated ellagitannin. The structure was elucidated as macabarterin by NMR analysis, including HSQC and HMBC, and comparison with literature data. To our best knowledge, this type of ellagitannin is firstly isolated from katsura tree bark and has never been reported before in domestic plant sources. This compound can be used an important valuable index marker to distinct the species.

## ACKNOWLEDGEMENTS

This study was supported by the Basic Research Program for Forest Science of Korean Forest Service (No S211315L010130) and also partially supported by Kangwon National University.

## REFERENCES

Haslam, E. 1989. Plant polyphenols: Vegetable tannins revisited. Chemistry and Pharmacology of Natural Products. 90～146, University of Cambridge Oriental Publications.

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.

Khallouki, F., Haubner, R., Hull, W.E., Erben, G., Spiegelhalder, B., Bartsch, H., Owen, R.W. 2007. Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. Food and Chemical Toxicology (45): 472～485.

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

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

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.

Ngoumfo, R.M., Ngounou, G.E., Tchamadeu, C.V., Qadir, M.I., Mbazoa, C.D., Begum, A., Ngninzeko, F.N., Lontsi, D., Choudhary, M.I. 2008. Inhibitory Effect of Macabarterin, a Polyoxygenated Ellagitannin from *Macaranga barteri*, on Human Neutrophil Respiratory Burst Activity. American Chemical Society and American Society of Pharmacognosy. 71: 1906～1910.

Sakanaka, S., Kim, M. Taniguchi, M., Yamamoto, T. 1989. Antibacterial substances in Japanese green tea extract against Streptococcus mutans, a cariogenic bacterium. Agricultural and Biological Chemistry (53): 2307～2311.

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.

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

Xianbin, S., Gemma, L.T., Warren, R.J.D.G., David, S.S., Richard, J.S., David, R.S. 2009. Synthesis of Biaryl-Containing Medium-Ring Systems by Organocuprate Oxidation: Applications in the Total Synthesis of Ellagitannin Natural Products. Synthesis No.22, pp. 3880～3896.

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).