Mokchae Konghak 37(2) : 164~170, 2009

Phenolic Compounds from the Bark of *Acer barbinerve* Max.*1

Dong-Joo Kwon*2 and Young-Soo Bae*2†

ABSTRACT

The bark of *Acer barbinerve* was extracted with 70% aqueous acetone and the organic extracts were concentrated to small volume using rotary evaporator and then fractionated successively with *n*-hexane, dichloromethane, ethyl acetate and water. The chromatographic separation of ethyl acetate soluble fraction led to the isolation of five phenolic compounds. By means of spectroscopic method, the structures of these compounds were identified to methyl gallate (**1**), methyl gallate-4-O-β-D-glucose (**2**), (+)-catechin (**3**), (-)-epicatechin (**4**) and (-)-epicatechin-3-O-gallate (**5**). These compounds (**1**-**5**) have not been reported in this plant yet.

Keywords : *Acer barbinerve*, phenolic compounds, methyl gallate, methyl gallate-4-O-β-D-glucose

1. INTRODUCTION

So far more than 200 species of *Acer* (Aceraceae) was found and widely distributed in the Northern hemisphere, especially in Chinese region (Ji *et al*., 1992). In Korea, the genus *Acer* comprises 15 species and these are main maple trees in mountain. The leaf, branch, and root of some species in the genus have been used in folk medicine for the treatment of arthralgia and fracture. *Acer barbinerve* Max. is indigenous to Korea and China, which is around 30 feet in height with a rare shrub (Kim *et al*., 1998a).

In genus *Acer* a variety of natural compounds were identified in the tissues of xylem, leaf and bark. In previous researches diarylheptanoids and cyclic diarylheptanoids were isolated form *A. nikoense* (Morikawa *et al*., 2003; Akihisa *et al*., 2006), tannins from *A. ginnala* and *A. saccharum* (Hatano *et al*., 1990), and flavonoids from *A. cissifolium* and *A. okamotoanum* (Aritomi, 1964; Kim *et al*., 1998b). However, no phytochemical and biological studies on natural compound isolated from *A. barbinerve* were reported yet.

In this study we have tried to isolate phenolic compounds from the bark of *A. barbinerve* by column chromatographic method followed by organic solvent extraction, and their chemical structures were identified by instrumental analy-

*1 Received on January 12, 2009; accepted on March 3, 2009
*2 Department of Wood Science & Engineering, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 200-701, Korea
† Corresponding author : Young-Soo Bae (e-mail: bae@kangwon.ac.kr)

sis using FAB-MS and ^{1}H, ^{13}C-NMR.

2. MATERIALS and METHODS

2.1. General

^{1}H- and ^{13}C-NMR spectra were measured with a Bruker DPX 400 (Germany) spectrometer in CD_3OD operated at 400 MHz and 100 MHz, respectively. Positive FAB-MS were recorded on a Micromass Autospec M363 (England) spectrometer using *m*-nitro benzyl alcohol (NBA) as a matrix. Column chromatograhic isolation was carried out on Sephadex LH-20 (25～100 μm, Sigma, Sweden). TLC analyses were performed on DC-Plastikfolien Cellulose F (Merck, Germany) plates and developed with TBAW (*t*-BuOH-HOAc-H_2O (3 : 1 : 1, v/v/v)) and 6% aqueous HOAc. Spots were detected under UV (254 and 365 nm) radiation and by spraying with vanillin (vanillin-EtOH-H_2SO_4 (15 : 250 : 2.5, w/v/v) and 1% methanolic $FeCl_3$, followed by heating.

2.2. Plant Materials

The bark of *Acer barbinerve* was collected at Hwacheon, Gangwon, Korea in June 2008 and it was identified by Professor Wan-Geun Park of the Department of Forestry. A voucher specimen has been deposited at the herbarium in the Department of Wood Science and Engineering, Kangwon National University.

2.3. Extraction and Isolation

The bark of *A. barbinerve* (3.5 kg) was extracted with 70% aqueous acetone at room temperature for 5 days. After removal of bark tissues by filtration the organic extracts were concentrated to small volume, which was then suspended in H_2O (1 ℓ), and partitioned successively with *n*-hexane (2 ℓ), dichloromethane (DCM; (2.5 ℓ)) and ethyl acetate (EtOAc; (4 ℓ)). The each fraction was concentrated *in vacuo* to obtain *n*-hexane fraction (8.4 g), DCM fraction (14.3 g), EtOAc fraction (150.7 g), and H_2O fraction (180.6 g), respectively.

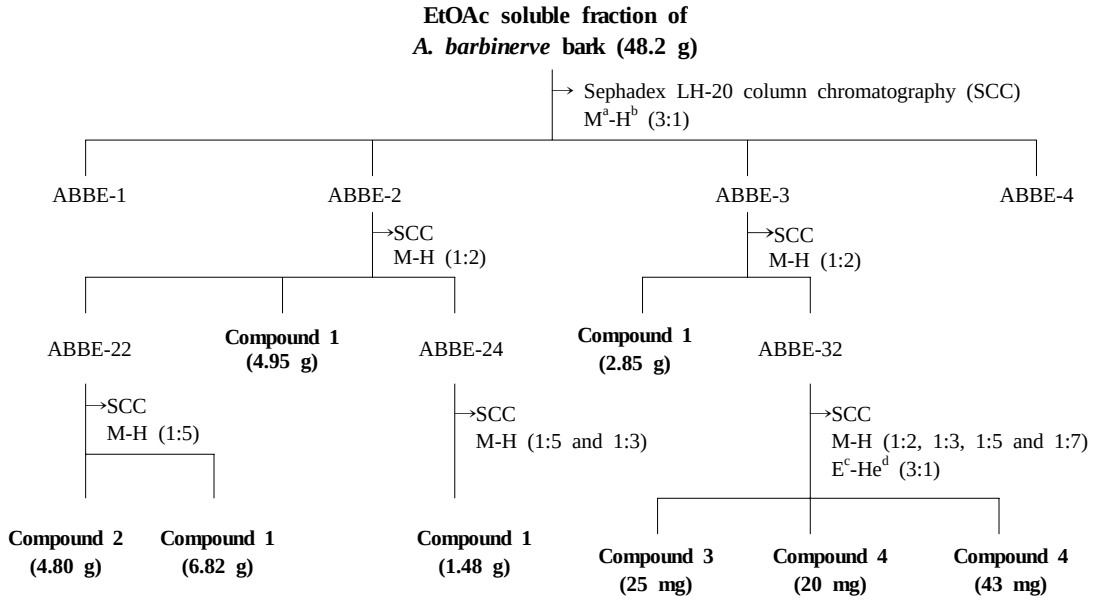
EtOAc soluble fraction (48.2 g) was separated by column chromatography (5 × 60 cm) packed with Sephadex LH-20 and then eluted with MeOH-H_2O (3 : 1, v/v; 2 ℓ) to obtain 4 fractions (ABBE 1-4). ABBE 2 and 3 fractions were further subjected to Sephadex LH-20 column and then eluted with MeOH-H_2O (1 : 2, v/v; 1 ℓ) to separate compound **1** (7.8 g; 0.22%). ABBE 22 and 24 fractions were rechromatographed using MeOH-H_2O (1 : 3 and 1 : 5, v/v; 1 ℓ) to yield compounds **1** (8.3 g; 0.24%) and **2** (4.8 g; 0.14%). ABBE 32 fraction was purified by repeated Sephadex LH-20 column chromatography eluted with EtOH-hexane (3 : 1, v/v; 0.8 ℓ) and MeOH-H_2O (1 : 2, 1 : 3, 1 : 5 and 1 : 7, v/v; 1 ℓ) to separate compounds **3** (25 mg; >0.001%), **4** (20 mg; >0.001%) and **5** (43 mg; >0.001%) (Fig. 1).

2.3.1. Methyl Gallate (1)

Brown amorphous powder, R_f 0.76 (TBAW) and 0.34 (6% HOAc). Positive FAB-MS : *m/z* 185 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 3.82 (3H, *s*, OCH_3), 7.05 (2H, *s*, H-2,6). ^{13}C-NMR (400 MHz, δ, CD_3OD) : 52.32 (OCH_3), 110.06 (C-2,6), 121.47 (C-1), 139.79 (C-4), 146.54 (C-3,5), 169.06 (C-7).

2.3.2. Methyl gallate-4-O-β-D-glucose (2)

Brown amorphous powder, R_f 0.48 (TBAW) and 0.64 (6% HOAc). Positive FAB-MS : *m/z* 347 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 3.35～3.82 (6H, *m*, H-2′, 3′, 4′, 5′, 6a′, 6b′), 3.85 (3H, *s*, OCH_3), 4.69 (1H, *d*, J = 7.9 Hz, H-1′), 7.05 (2H, *s*, H-2,6). ^{13}C-NMR (400 MHz,



[a]M: MeOH, [b]H: H_2O, [c]E: EtOH, [d]He: *n*-hexane

Fig. 1. Column chromatography procedure on the EtOAc soluble fraction of *A. barbinerve* bark.

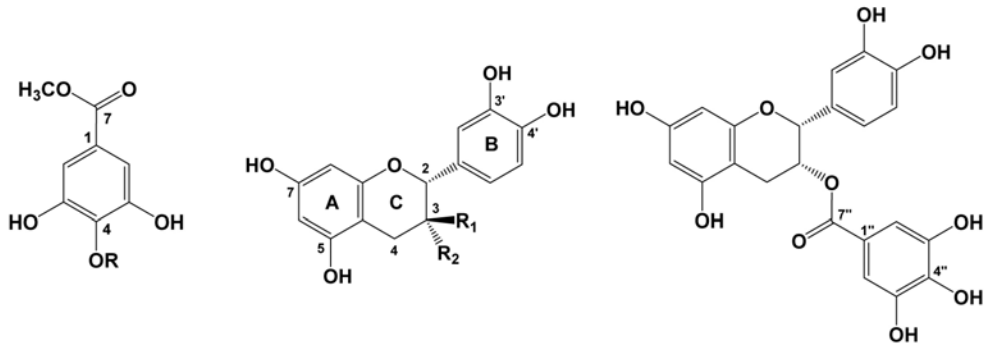
δ, CD_3OD) : 52.67 (OCH_3), 61.89 (C-6′), 70.63 (C-4′), 75.14 (C-2′), 77.66 (C-3′), 78.57 (C-5′), 107.32 (C-1′), 110.18 (C-2,6), 128.46 (C-1), 138.50 (C-4), 151.66 (C-3,5), 168.20 (C-7).

2.3.3. (+)-catechin (3)

Yellow amorphous powder, R_f 0.53 (TBAW) and 0.33 (6% HOAc). Positive FAB-MS : *m/z* 291 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 2.50 (1H, *dd*, *J* = 8.1 Hz and 16.1 Hz, H-4ax), 2.84 (1H, *dd*, *J* = 5.4 Hz and 16.1 Hz, H-4eq), 3.97 (1H, *m*, H-3), 4.57 (1H, *d*, *J* = 7.5 Hz, H-2), 5.86 (1H, *d*, *J* = 2.4 Hz, H-6), 5.93 (1H, *d*, *J* = 2.3 Hz, H-8), 6.72 (1H, *dd*, *J* = 1.7 Hz and 8.1 Hz, H-6′), 6.76 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.84 (1H, *d*, *J* = 1.8 Hz, H-2′). ^{13}C- NMR (100 MHz, δ, CD_3OD) : 28.55 (C-4), 68.84 (C-3), 82.88 (C-2), 95.53 (C-8), 96.32 (C-6), 100.85 (C-10), 115.28 (C-2′), 116.12 (C-5′), 120.08 (C-6′), 132.24 (C-1′), 146.26 (C-3′), 146.28 (C-4'), 156.95 (C-9), 157.61 (C-5), 157.86 (C-7).

2.3.4. (-)-epicatechin (4)

Yellow amorphous powder, R_f 0.37 (TBAW) and 0.31 (6% HOAc). Positive FAB-MS : *m/z* 291 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 2.73 (1H, *dd*, *J* = 2.8 Hz and 16.8 Hz, H-4ax), 2.86 (1H, *dd*, *J* = 4.5 Hz and 16.8 Hz, H-4eq), 4.17 (1H, *m*, H-3), 4.81 (1H, *s*, H-2), 5.91 (1H, *d*, *J* = 2.3 Hz, H-6), 5.94 (1H, *d*, *J* = 2.3 Hz, H-8), 6.75 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.80 (1H, *dd*, *J* = 1.8 Hz and 8.3 Hz, H-6′), 6.97 (1H, *d*, *J* = 1.7 Hz, H-2′). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 29.31 (C-4), 67.54 (C-3), 79.92 (C-2), 95.93 (C-8), 96.43 (C-6), 100.11 (C-10), 115.37 (C-2′), 115.93 (C-5′), 119.44 (C-6′), 132.34 (C-1′), 145.83 (C-3′), 145.99 (C-4′), 157.42 (C-9), 157.73 (C-5), 158.05 (C-7).



1 R = H
2 R = β-D-glucose
3 R_1 = OH, R_2 = H
4 R_1 = H, R_2 = OH
5

Fig. 2. The chemical structures of compounds 1-5 isolated from *A. barbinerve* bark.

2.3.5. (-)-epicatechin-3-O-gallate (5)

Pale yellow amorphous powder, R_f 0.47 (TBAW) and 0.35 (6% HOAc). Positive FAB-MS : *m/z* 443 $[M+H]^+$. ^{1}H-NMR (400 MHz, δ, CD_3OD) : 2.85 (1H, *dd*, *J* = 2.2 Hz and 17.4 Hz, H-4ax), 3.00 (1H, *dd*, *J* = 4.5 Hz and 17.4 Hz, H-4eq), 5.03 (1H, *s*, H-2), 5.53 (1H, *m*, H-3), 5.96 (2H, *s*, H-6,8), 6.70 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.81 (1H, *dd*, *J* = 2.0 Hz and 8.3 Hz, H-6′), 6.93 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.95 (2 H, *s*, H-2″, 6″). ^{13}C-NMR (100 MHz, δ, CD_3OD) : 25.47 (C-4), 68.58 (C-3), 77.24 (C-2), 94.50 (C-8), 95.15 (C-6), 98.80 (C-10), 108.82 (C-2″, 6″), 113.72 (C-2′), 114.61 (C-5′), 117.99 (C-6′), 120.08 (C-1″), 130.07 (C-1′), 138.41 (C-4″), 144.54 (C-3′), 144.92 (C-3″, 5″), 145.28 (C-4′), 155.83 (C-9), 155.88 (C-5), 156.47 (C-7), 166.21 (C-7″).

3. RESULTS and DISCUSSION

As shown in Fig. 2, 5 phenolic compounds were isolated by repeated Sephadex LH-20 column chromatography of EtOAc soluble fraction, which was fractionated from 70% aqueous acetone extracts of *A. barbinerve* bark.

Compounds **1** and **2** were obtained as a brown amorphous powder, and gave greenish blue color with spraying of ferric chloride (1% methanolic $FeCl_3$) on TLC. The ^{1}H-NMR spectrum of **1** showed a singlet signal at δ 7.05 due to a pair of symmetric galloyl protons, and one methoxyl protons at δ 3.82. The ^{13}C-NMR spectrum of **1** exhibited a carbonyl carbon at δ 169.06, two pairs of symmetric galloyl carbons at δ 110.06 (C-2,6) and δ 146.54 (C-3,5), and one methoxyl carbon at δ 52.32. The ^{1}H- and ^{13}C-NMR spectrum of **2** were very similar to those of **1** except the presence of glucose moiety. The anomeric proton of glucose indicated a doublet signal at δ 4.69 (*J* = 7.9 Hz), the rest protons gave multiple signals between at δ 3.35 and 3.82. The ^{13}C-NMR spectrum of **2**, the typical glucose signals were observed at δ 107.32 (C-1′), δ 75.14 (C-2′), δ 77.66 (C-3′), δ 70.63 (C-4′), δ 78.57 (C-5′) and δ 61.89 (C-6′). Thus, the structures of **1** and **2** were identified as methyl gallate (Lee and Jeong, 2005; Kim *et al*., 2008) and methyl gallate-4-O-β-D-glucose (Lee *et al*., 2002), respectively.

Compounds **3** and **4** possess two chiral centers, located at C-2 and C-3 position of heterocyclic C-ring. In the ^{13}C-NMR spectrum, C-2 carbon signals of **3** and **4** at δ 82.88 and 79.92

were characteristic of the *trans*- and *cis*-from, respectively (Agrawal, 1989). Accordingly, the structures of **3** and **4** were determinated as (+)-catechin and (-)-epicatechin (Harbone and Mabry, 1982).

Compound **5** was obtained as a pale yellow amorphous powder, and gave red color with spraying of vanillin on TLC. The molecular formula $C_{22}H_{18}O_{10}$ was supported by molecular ion peak *m/z* 443 $[M+H]^+$ from FAB-MS spectrum. The structure of **5** was similar to that of **4**, except for C-3 position difference of the hydroxy group located on the gallic acid moiety. Compared to ^{1}H-NMR spectra data of **4**, one singlet signal of aromatic protons of gallic acid moiety was observed at δ 6.95 (H-2″, 6″), the downfield shift of H-3 and H-2 signals at δ 5.53 and δ 5.03. In the ^{13}C-NMR spectrum of **5**, the characteristic signals at δ 108.82 (C-2″, 6″), δ 144.92 (C-3″, 5″) and δ 166.21 (C-7″) indicated the present of gallic acid moiety. The two upfield and one downfield signals at δ 25.47, δ 77.24 and δ 68.58 were attributable to the heterocyclic C-ring carbons C-4, C-2 and C-3, respectively (Davis *et al*., 1996). The remaining ^{1}H- and ^{13}C-NMR spectra data of **5**, phloroglucinol A-ring and catechol B-ring, were same as **4**. According to the above data, compound **5** was identified as (-)-epicatechin-3-O-gallate (Alessandra *et al*., 2003; Wan *et al*., 2004).

To our knowledge, these compounds (**1**-**5**) are reported from this plant for the frist time. Methyl gallate (**1**) and its glucoside (**2**) were the major isolated compound from *A. barbinerve* bark. Methyl gallate (**1**) has been previously isolated from several other plants including the genus *Acer* and is a potent and highly specific inhibitor of herpes simplex virus (Kane *et al*., 1988). It has shown antioxidant activity (Hsieh *et al*., 2004), antimicrobial activity (Penna *et al*., 2001), and cancer chemopreventive effects (Nakamura *et al*., 2002). Methyl gallate-4-O-β-D-glucose (**2**) has been isolated from *Acer tegmentosum* (Park *et al*., 2006) and *Carpinus cordata* (Lee *et al*., 2002). (+)-catechin (**3**) and its derivatives, such as (-)-epicatechin (**4**), (-)-epicatechin-3-O-gallate (ECG) (**5**) and (-)-epigallocatechin-3-O-gallate (EGCG), are polyphenolic antioxidant plant metabolites. These compounds are abundant in teas derived from the tea-plant *Camellia sinensis*, and a variety of bioactivities are known, with antioxi-dative, anticancer, antiallergic, anti-inflammatory, antiangiogenicand hypolipidemic properties demonstrated (Dona *et al*., 2003; Hakim *et al*., 2003; Lee *et al*., 2005).

4. CONCLUSIONS

Phenolic compounds isolated from the bark of *Acer barbinerve* were elucidated by ^{1}H-NMR, ^{13}C-NMR and FAB-MS to methyl gallate (**1**) (16.1 g, 0.46%), methyl gallate-4-O-β-D-glucose (**2**) (4.8 g, 0.14%), (+)-catechin (**3**) (25 mg, >0.001%), (-)-epicatechin (**4**) (20 mg, >0.001%) and (-)-epicatechin-3-O-gallate (**5**) (43 mg, >0.001%). Although these compounds were found in other plant tissues, it could be meaningful that these compounds (**1**-**5**) were first identified from *Acer barbinerve*. Furthermore, methyl gallate (**1**) and its glucoside (**2**), due to their enormous amounts, could be functioned to chemotaxonomic makers for *A. barbinerve*.

ACKNOWLEDGEMENT

This study was partially supported by The Basic Research Program for Forest Science funded by the Korea Forest Service. We are also grateful to Dr. Ji-Sook Ryu, Central Laboratory of Kangwon National University, for measuring the NMR spectra.

REFERENCES

1. Agrawal, P. K. 1989. Carbon-13 NMR of flavonoids, Elsevier. pp. 437～445.
2. Akihisa, T., Y. Taguchi, K. Yasukawa, H. Tokuda, H. Akazawa, T. Suzuki, and Y. Kimura. 2006. Acerogenin M, a cyclic diarylheptanoid, and other phenolic compounds from *Acer nikoense* and their anti-inflammatory and anti-tumor-promoting effects. Chemical & Pharmaceutical Bulletin 54(5): 735～739.
3. Alessandra, B., P. Matteo, S. Rokia, S. Haby, M. Ivano, and P. Cosimo. 2003. Chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocarya birrea* (Anacardiaceae) leaves. Journal of Agricultural and Food Chemistry 51(23): 6689～6695.
4. Aritomi, M. 1964. Chemical constituents in Aceraceous plants. III. Flavonoid constituents in leaves of *Acer cissifolium*. Chemical & Pharmaceutical Bulletin 12(7): 841～843.
5. Davis, A. L., C. Ya, A. P. Davies, and J. R. Lewis. 1996. 1 H and 13C NMR assignments of some green tea polyphenols. Magnetic Resonance in Chemistry 34(11): 887～890.
6. Dona, M., I. Dell'Aica, F. Calabrese, R. Benelli, M. Morini, A. Albini, and S. Garbisa. 2003. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. Journal of Immunology 170(8): 4335～4341.
7. Hakim, I. A., R. B. Harris, S. Brown, H. H. Chow, S. Wiseman, S. Agarwal, and W. Talbot. 2003. Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. Journal of Nutrition 133: 3303S～3309S.
8. Harbone, J. B. and T. J. Mabry. 1982. The flavonoids: advance in research, Chapman and Hall Ltd. pp. 421～426.
9. Hatano, T., S. Hattori, Y. Ikeda, T. Shingu, and T. Okuda. 1990. Tannins of Aceraceous plants. Part II. Gallotannins having a 1,5-anhydro-D-glucitol core and some ellagitannins from *Acer* species. Chemical & Pharmaceutical Bulletin 38(7): 1902～5.
10. Hsieh, T. J., T. Z. Liu, Y. C. Chia, C. L. Chern, F. J. Lu, M. C. Chuang, S. Y. Mau, S. H. Chen, Y. H. Syu, and C. H. Chen. 2004. Protective effect of methyl gallate from *Toona sinensis* (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. Food and Chemical Toxicology 42(5): 843～850.
11. Ji, S. B., M. Yokoi, N. Saito, and L. S. Mao. 1992. Distribution of anthocyanins in Aceraceae leaves. Biochemical Systematics and Ecology 20(8): 771～781.
12. Kane, C. J., J. H. Menna, and Y. C. Yeh. 1988. Methyl gallate, methyl-3,4,5-trihydroxy-benzoate, is a potent and highly specific inhibitor of herpes simplex virus *in vitro*. I. Purification and characterization of methyl gallate from *Sapium sebiferum*. Bioscience Reports 8: 85～94.
13. Kim, C. M., M. K. Shin, D. K. An, and K. S. Lee. 1998a. Dictionary of traditional medicines, Jung-Dam Publishing House. Seoul. pp. 3915～3916.
14. Kim, J. I., H. H. Kim, S. G. Kim, K. T. Lee, I. H. Ham, and W. K. Whang. 2008. Antioxidative compounds from *Quercus salicina* Blume stem. Archives of Pharmacal Research 31(3): 274～278.
15. Kim, H. J., E. R. Woo, C. G. Shin, and H. K. Park. 1998b. A new flavonol glycoside gallate ester from *Acer okamotoanum* and its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. Journal of Natural Products 61(1): 145～148.
16. Lee, H. Y. and H. S. Jeong. 2005. Isolation and identification of antimicrobial substance from *Canavalia gladiata*. Food Science and Biotechnology 14(2): 268～274.
17. Lee. J. S., H. J. Kim, H. K. Park, and Y. S. Lee. 2002. New diarylheptanoids from the stems of *Carpinus cordata*. Journal of Natural Products 65(9): 1367～1370.
18. Lee, Y. S., C. H. Han, S. H. Kang, S. J. Lee, S. W. Kim, O. R. Shin, Y. C. Sim, S. J. Lee, and Y. H. Cho. 2005. Synergistic effect between catechin and ciprofloxacin on chronic bacterial prostatitis rat model. International Journal of

Urology 12(4): 383～389.
19. Morikawa, T., J. Tao, I. Toguchida, H. Matsuda, and M. Yoshikawa. 2003. Structures of new cyclic diarylheptanoids and inhibitors of nitric oxide production from Japanese folk medicine *Acer nikoense*. Journal of Natural Products 66(1): 86～91.
20. Nakamura, E. S., F. Kurosaki, M. Arisawa, T. Mukainaka, J. Takayasu, M. Okuda, H. Tokuda, H. Nishino, and F. Pastore. 2002. Cancer chemopreventive effects of a Brazilian folk medicine, Juca, on *in vivo* two-stage skin carcinogenesis. Journal of Ethnopharmacology 81(1): 135～137.
21. Park, K. M., M. C. Yang, K. H. Lee, S. U. Choi, and K. R. Lee. 2006. Cytotoxic phenolic constituents of *Acer tegmentosum* Maxim. Archives of Pharmacal Research 29(12): 1086～1090.
22. Penna, C., S. Marino, E. Vivot, M. C. Cruanes, J. D. Munoz, J. Cruanes, G. Ferraro, G. Gutkind, and V. Martino. 2001. Antimicrobial activity of Argentine plants used in the treatment of infectious diseases. Isolation of active compounds from *Sebastiania brasiliensis*. Journal of Ethnopharmacology 77(1): 37～40.
23. Wan, S. B., D. Chen, Q. P. Dou, and T. H. Chan. 2004. Study of the green tea polyphenols catechin-3-gallate (CG) and epicatechin-3-gallate (ECG) as proteasome inhibitors. Bioorganic & Medicinal Chemistry 12(13): 3521～3527.