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A new flavonoid from the bark of *Ulmus pumila* L

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ABSTRACT

A new flavonoid (**6**), together with eight (**1–5** and **7–9**) known flavonoids, were isolated from the *n*-butyl alcohol soluble portion of the EtOH extract of *Ulmus pumila* L. The chemical structures of the compounds were determined by using spectroscopic methods and further supported by comparison with previously literature values. Among them, flavonoids **4, 6 and 9** were isolated for the first time from the family Ulmaceae. Furthermore, the chemotaxonomic significance of the isolates was also discussed.

1. Subject and source

The genus *Ulmus* contains more than 40 species, which are naturally distributed throughout the northern hemisphere in Eurasia, North America, Central America, and Northern Africa (MartínBenito et al., 2005). In China, there are about 25 species of *Ulmus* plants that are widely distributed. *Ulmus pumila* L. has been reported to have antioxidant (Cho et al., 2016), anti-inflammatory (Lee et al., 2018), antimicrobial (You et al., 2013) and anti-adipogenic activities (Ghosh et al., 2012).

In the present study, the bark of *U. pumila* was collected in the Changbai Mountain area, Jilin Province, China, in July 2016 and was identified by Professor Hui-zi Lv (College of Pharmacy, Yanbian University, Jilin). A voucher specimen (YB-YSP-180704) was deposited at the College of Pharmacy, Yanbian University.

2. Previous work

Previous phytochemical investigations on genus *Ulmus* have identified diverse types of compounds, which include flavonoids (Kwon et al., 2011), terpenoids (Wang et al., 2006), lignans (Zheng et al., 2010a), coumarins (Kwon et al., 2011) and glycosides (Zheng et al., 2011).

In our previous studies, we have led to the isolation of triterpenes, flavonoids, lignans (Zheng et al., 2010a, 2010b; Yang et al., 2011) and catechin glycosides (Zheng et al., 2014) from *Ulmus davidiana*, which is a different species of the same genus. Among these, the flavonoids and triterpenes appeared to be the major classes of compounds of the genus *Ulmus*. But, there are few reports about the constitutes in the bark of *U. pumila,* which one also have important chemotaxonomical significance.

3. Present study

The air-dried bark of *U. pumila* (9.0 kg) was extracted with 75% ethanol (3 × 60 L) by reflux.

The crude extract (1.6 kg) was suspended with distilled water and successively partitioned into petroleum ether (PE, 3 × 1.4 L), ethyl acetate (EtOAc, 3 × 1.4 L) and *n*-butyl alcohol (*n*-BuOH, 3 × 1.4 L). The *n*-BuOH extract (78.0 g) was subjected to silica gel column chromatography (CC) using a gradient elution of CH_2Cl_2–MeOH (100:1–0:100, v/v) to afford 12 fractions (Fr. 1–Fr. 12). Fr. 5 (1.0 g) was applied to silica gel CC using CH_2Cl_2–MeOH (100:1–0:100, v/v) as the mobile phase to give eight subfractions (Fr.5-1–Fr.5-8). Fr.5-3 was purified over Sephadex LH-20 gel with isocratic elution (100% MeOH) to afford compound **1** (83.9 mg). Fr. 6 (8.6 g) was fractionated by CC over silica gel with CH_2Cl_2–MeOH (50:1–3:1, v/v) gradient elution to give 10 subfractions (Fr. 6-1–Fr. 6-10). Fr. 6-4 was purified using a Sephadex LH-20 column with 100% MeOH to afford compound **2** (286.1 mg). Fr. 6-7 was further separated into five subfractions (Fr. 6-7-1–Fr. 6-7-5) by silica gel CC with gradient elution of CH_2Cl_2–MeOH (30:1–1:1, v/v). Fr. 6-7-2 was applied to a reverse-phase (RP) CC with MeOH–H_2O (1:9–1:0, v/v) gradient elution to yield compound **3** (18.0 mg) and compound **4** (6.2 mg) successively. Fr. 6-8 was separated successively by a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1, v/v) and a RP column with MeOH–H_2O (20:1–1:0, v/v) to give compound **5** (40.0 mg) and compound **6** (16.0 mg). Fr. 8 (6.6 g) was subjected to a RP column with MeOH–H_2O (1:9–1:0, v/v) to give 10 subfractions (Fr. 8-1–Fr. 8-10). Fr. 8-2 was further separated by a RP column with

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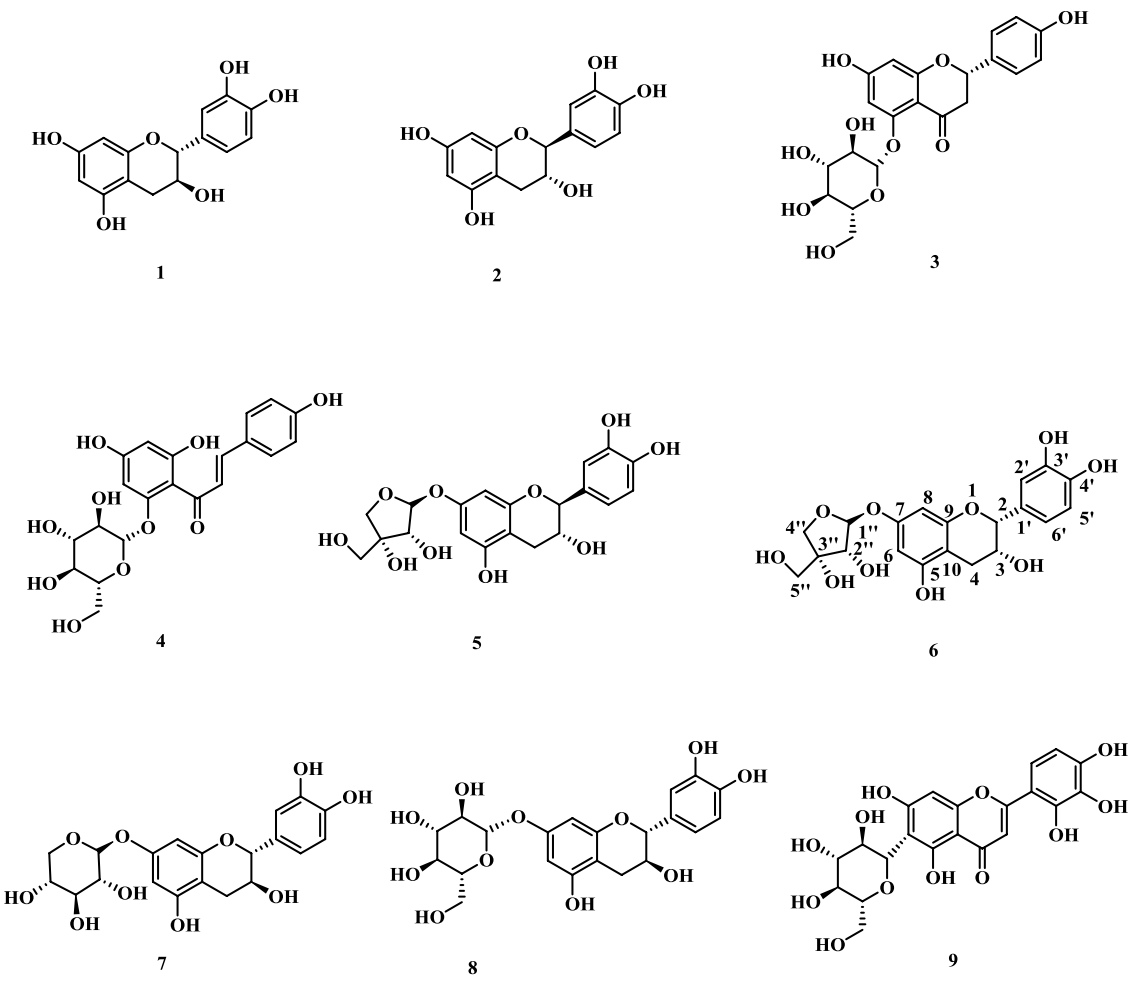

Fig. 1. Chemical structures of compounds **1–9** isolated from *U. pumila*.

MeOH–H_2O (1:8–1:0, v/v) and purified by a Sephadex LH-20 column with MeOH to give compound **7** (40.0 mg). Fr. 9 (3.5 g) was chromatographed over a silica gel column using a solvent system of CH_2Cl_2–MeOH (50:1–1:1, v/v) to obtain eight subfractions (Fr. 9-1–Fr. 9-8). Fr. 9-2 was further separated by a silica gel column with CH_2Cl_2–MeOH (40:1–1:1, v/v) and a RP with H_2O–MeOH (7:1–0:1, v/v) to obtain compound **8** (8.0 mg). Fr. 9-3 was purified by a Sephadex LH-20 column with MeOH to give compound **9** (14.0 mg).

One new flavonoid (**6**) and eight known flavonoids (**1–5**, **7–9**) were isolated from the *n*-BuOH extract of *U. pumila*. The structures of isolated compounds were elucidated on the basis of spectrometric analysis (^{1}H-NMR, ^{13}C-NMR, 2D NMR, optical rotation experiments and ESI-MS) and by comparison with the literature data. The eight known compounds were identified as (+)-catechin (**1**) (Mei et al., 2016), (−)-catechin (**2**) (Zheng et al., 2011), (2S)-(−)-naringenin-5-O-*β*-D-glucopyranoside (**3**) (Wang et al., 2009), isosalipurposide (**4**) (Ghouila et al., 2012), (−)-catechin-7-O-*β*-D-apiofuranoside (**5**) (Jung et al., 2010), catechin-7-O-*β*-D-xylopyranoside (**7**) (Pan et al., 1994), catechin-7-O-*β*-D-glucopyranoside (**8**) (Zerbib et al., 2018) and 2′-hydroxyisoorientin (**9**) (Norbaek et al., 2000) (Fig. 1).

Compound **6** was obtained as a yellowish-brown powder and its molecular formula $C_{20}H_{22}O_{10}$ was determined by ESI-MS. The ^{1}H-NMR spectrum of **6** displayed signals for three aromatic protons at δ_H 6.98 (d, J = 1.7 Hz, H-2′), 6.80 (dd, J = 8.1, 1.7 Hz, H-6′) and 6.76 (d, J = 8.1 Hz, H-5′) suggesting the presence of an ABX spin system (Table 1). Two aromatic proton signals at δ_H 6.14 (d, J = 3.8 Hz, 2H, H-6,-8) showed H-6 and H-8 were *meta* coupled. It also exhibited four aliphatic protons characteristic of epicatechin at δ_H 4.82 (br s, H-2), 4.19 (m, H-3), 2.88 (dd, J = 16.8, 4.4 Hz, H-4a) and 2.74 (dd, J = 16.8, 2.6 Hz, H-4b) (Mei et al., 2016). The epicatechin group based on the H-4 shift at δ_H 2.88 (H-4a) and 2.74 (H-4b), which was significantly

Table 1
^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) of compound **6** in CD_3OD (δ in ppm, J in Hz).

NO.	δ_H	δ_C
2	4.82 (br s)	80.29
3	4.19 (m)	67.29
4	2.74 (dd, J = 16.8, 2.6) 2.88 (dd,J = 16.8, 4.4)	29.27
5		157.31
6	6.14 (d, J = 3.8)	97.40
7		158.01
8	6.14 (d, J = 3.8)	97.17
9		157.95
10		102.51
1′		132.15
2′	6.98 (d,J = 1.7)	115.28
3′		145.83
4′		145.95
5′	6.76 (d, J = 8.1)	115.90
6′	6.80 (dd, J = 8.1, 1.7)	119.36
1″	5.49 (d, J = 2.9)	108.72
2″	4.14 (d, J = 2.9)	78.27
3″		79.94
4″	3.85 (d, J = 9.7) 4.09 (dd, J = 9.7, 4.2)	75.40
5″	3.62 (m)	64.95

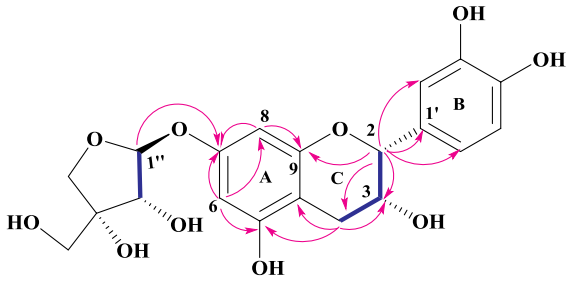

Fig. 2. Important HMBC and ^{1}H–^{1}H COSY correlations for compound **6**.

different from the H-4 of catechin [δ_H 2.86 (H-4a), 2.54 (H-4b)] (Souza et al., 2008). Furthermore, the optical rotation, [α]22 D −27.4° (*c* 0.02, MeOH) evidenced the 2*R*, 3*R* absolute configuration which suggested that compound **6** has the same absolute configuration with (−)-epicatechin (Seto et al., 1997). In addition, an anomeric proton was observed at δ_H 5.49 (d, *J* = 2.9 Hz, H-1″), as well as four aliphatic signals at δ_H 4.14 (d, *J* = 2.9 Hz, H-2″), 4.09 (m, H-4″a), 3.85 (d, *J* = 9.7 Hz, H-4″b) and 3.62 (m, H-5″) showed the presence of a D-apiofuranose (Iwamoto et al., 2012) and the mode of sugar linkage were assigned to be *β* from the coupling constant of anomeric proton of apiofuranoside [δ_H 5.49 (d, *J* = 2.9 Hz, H-1″)] (Iwamoto et al., 2012). The ^{13}C-NMR spectrum showed twenty carbon signals and indicated the presence of the apiofuranose that was supported by the signals at δ_C 108.72 (C-1″), 79.94 (C-3″), 78.27 (C-2″), 75.40 (C-4″) and 64.96 (C-5″) (Zheng et al., 2011). The remaining fifteen carbon signals corresponded to those of epicatechin (Mei et al., 2016) as shown by the characteristic signals at δ_C 158.01 (C-7), 157.95 (C-9), 157.31 (C-5), 145.95 (C-4′), 145.83 (C-3′), 132.15 (C-1′), 119.36 (C-6′), 115.90 (C-5′), 115.28 (C-2′), 102.51 (C-10), 97.40 (C-6), 97.17 (C-8), 80.29 (C-2), 67.29 (C-3) and 29.27 (C-4). The connectivities of ring B and ring C were confirmed by the HMBC correlations between H-2 with C-9, C-1′, C-2′ and C-6' (Fig. 2). The position of the sugar unit was determined by the HMBC correlation observed between its anomeric proton at δ_H 5.49 (H-1″) and the aromatic carbon signal at δ_C 158.01 (C-7) (Fig. 2), which proved its location at C-7. The planar structure was determined by extensive analysis of ^{1}H–^{1}H–COSY and HMBC spectra (Fig. 2). Based on the above evidence, the structure of **6** therefore elucidated as (−)-epicatechin-7-O-*β*-D-apiofuranoside.

(−)-Epicatechin-7-O-*β*-D-apiofuranoside (**6**): yellowish-brown powder; [α]22 D = −27.4° (*c* 0.02, MeOH); UV λ_{max} MeOH (nm) (log *ε*): 278 (3.50), 215 (4.25), 204 (4.22); IR ν_{max} 3342, 2929, 2372, 1627, 1598, 1442, 1282, 1149, 1058, 1008, 827, 790 and 617 cm^{-1}; ^{1}H-NMR (CD_3OD, 300 MHz) and ^{13}C-NMR (CD_3OD, 75 MHz) data is shown in Table 1; ESI-MS: *m/z* = 423.1286 [M] + (calcd. for $C_{20}H_{23}O_{10}$, 423.1278).

4. Chemotaxonomic significance

In the present phytochemical study, nine flavonoid compounds were isolated from the bark of *U. pumila*, including six flavan-3-ols and their glycosides (**1**, **2**, and **5–8**), one dihydroflavone glycoside (**3**), one chalcone glycoside (**4**), and one flavone glycoside (**9**), which could be considered as typical metabolites of *U. pumila*. These findings enrich the chemical diversity of *U. pumila* and provide evidence for further chemotaxonomic studies.

To our knowledge, compounds (2*S*)-(−)-naringenin-5-O-*β*-D-glucopyranoside (**3**), isosalipurposide (**4**), (−)-catechin-7-O-*β*-D-apiofuranoside (**5**), (−)-epicatechin-7-O-*β*-D-apiofuranoside (**6**), catechin-7-O-β-D-xylopyranoside (**7**), catechin-7-O-*β*-D-glucopyranoside (**8**), and 2′-hydroxyisoorientin (**9**) were isolated from *U. pumila* for the first time. Among them, compounds **2**, **3**, **5**, **7**, and **8** have previously been isolated from *U. davidiana*, a different species of the same genus (Zheng et al., 2010a, 2014). Therefore, the presence of **2**, **3**, **5**, **7**, and **8** indicated the close phylogenetic relationship between these *Ulmus* species. Compounds **4**, **6**, and **9** were newly identified in the genus *Ulmus* and the family Ulmaceae.

(+)-Catechin (**1**) and (−)-catechin (**2**) were first isolated from green tea (Bradfield et al., 1947). These compounds are also common in *Ulmus* species (Cho et al., 2016; Zheng et al., 2010a). Compounds **3** and **4** were first identified in *Salix purpurea* L. (Bridel, 1931; Charaux and Rabate, 1993). In this study, **3** was isolated from *U. davidiana* (So et al., 2019) and **4** from *U. pumila*. These findings demonstrate the correlation between the *Ulmus* and *Salix* genera. Compound **5** was first reported in *U. davidiana* (Jung et al., 2007). Furthermore, compound **5** was only found in the genus *Ulmus*. Therefore, compound **5** might be valuable for identifying plants from the genus *Ulmus*. Compound **6**, a new flavonoid isolated and reported from the family Ulmaceae for the first time, can be considered a characteristic component of *U. pumila*. Compounds **7** and **8** were previously reported in *Betula ermanii* (Hiroyuki et al., 1995) and *Schizandra nigra* (Masako et al., 1977) for the first time, respectively, and have also been obtained from *Ulmus* species, including *U. davidiana* (Lee et al., 2008). Identification of the same compounds in *U. davidiana* and *U. pumila* confirmed their close relationship. Compound **9** was first isolated from the leaf of *Hordeum vulgare* (Norbk et al., 2003). Herein, compound **9** was identified from an organism in the genus *Ulmus* for the first time. All of these compounds can help extend the phytochemical knowledge of the genus *Ulmus*.

In conclusion, (−)-catechin-7-O-*β*-D-apiofuranoside (**5**) and (−)-epicatechin-7-O-β-D-apiofuranoside (**6**) can be regarded as important chemotaxonomic markers of the genus *Ulmus*. The present study extends the phytochemical composition of the genus *Ulmus* and provides new chemotaxonomic markers for *U. pumila* and the genus *Ulmus*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2019.103956.

References

Bradfield, A.E., Penny, M., Wright, W.B., 1947. The catechins of green tea. J. Chem. Soc. 103, 3–5.
Bridel, Marc, 1931. Salipurposide presenting the physiological action of phlorhizin. Bull. Acad. Med. 106, 353–355.
Charaux, C., Rabate, J., 1993. The biochemical study of genus Salix. V. isosalipurposide. Compt. Rendus Acad. Sci. 196, 816–818.
Cho, M.L., Ko, S.B., Kim, J.M., Lee, O.H., Lee, D.W., Kim, J.Y., 2016. Influence of extraction conditions on antioxidant activities and catechin content from bark of *Ulmus pumila* L. Appl. Biol. Chem. 59, 329–336.
Ghosh, C., Chung, H.Y., Nandre, R.M., Lee, J.H., Jeon, T.I., Kim, I.S., Yang, S.H., Hwang, S.G., 2012. An active extract of *Ulmus pumila* inhibits adipogenesis through regulation of cell cycle progression in 3T3-L1 cells. Food Chem. Toxicol. 50, 2009–2015.
Ghouila, H., Meksi, N., Haddar, W., Mhenni, M.F., Jannet, H.B., 2012. Extraction, identification and dyeing studies of isosalipurposide, a natural chalcone dye from *Acacia cyanophylla* flowers on wool. Ind. Crop. Prod. 35, 31–36.
Hiroyuki, F., Tetsuya, S., Nobutoshi, T., 1995. Chemical evaluation of betula species in Japan. I. Constituents of *Betula ermanii*. Chem. Pharm. Bull. 43, 1937–1942.
Iwamoto, Y., Sugimoto, S., Harinantenaina, L., Matsunami, K., Otsuka, H., 2012. Entadosides A-D, triterpene saponins and a glucoside of the sulphur-containing amide from the kernel nuts of *Entada phaseoloides* (L.) Merrill. J. Nat. Med. 66, 321–328.
Jung, M.J., Heo, S.I., Wang, M.H., 2007. Reducing power and α-glucosidase inhibitory profiles of (-)-catechin and its glycoside. Saengyak Hakhoechi 38, 358–362.

Jung, M.J., Heo, S.I., Wang, M.H., 2010. HPLC analysis and antioxidant activity of *Ulmus davidiana* and some flavonoids. Food Chem. 120, 313–318.
Kwon, H., Kim, S.B., Park, K.H., Lee, M.W., 2011. Antioxidative and anti-inflammatory effects of phenolic compounds from the roots of *Ulmus macrocarpa*. Arch Pharm. Res. (Seoul) 34, 1459–1466.
Lee, G.Y., Jang, D.S., Kim, J., Kim, C.S., Kim, Y.S., Kim, J.H., Kim, J.S., 2008. Flavan-3-ols from *Ulmus davidiana* var. *japonica* with inhibitory activity on protein glycation. Planta Med. 74, 1800–1802.
Lee, J.H., Lee, Y.K., Choi, Y.R., Park, J., Jung, S.K., Chang, Y.H., 2018. The characterization, selenylation and anti-inflammatory activity of pecticpolysaccharides extracted from *Ulmus Pumila* L. Int. J. Biol. Macromol. 111, 311–318.
MartínBenito, D., Concepción García-Vallejo, M., Pajares, J.A., López, D., 2005. Triterpenes in elms in Spain. Can. J. For. Res. 35, 199–205.
Masako, T., Machiko, N., Kotaro, T., 1977. Studies on constituents of medicinal plants. XIX. Constituents of *Schizandra nigra* Max. (3). Chem. Pharm. Bull. 25, 3388–3390.
Mei, Q.X., Chen, X.L., Xia, X., Fang, Z.J., Zhou, H.B., Gao, Y.Q., Dai, W.B., Jiang, R.W., 2016. Isolation and chemotaxonomic significance of chemical constituents from *Rubus parvifolius*. Chin. Herb. Med. 8, 75–79.
Norbaek, R., Brandt, K., Kondo, T., 2000. Identification of flavone C-glycosides including a new flavonoid chromophore from barley leaves (*Hordeum vulgare* L.) by improved NMR techniques. J. Agric. Food Chem. 48, 1703–1707.
Norbk, R., Aaboer, D.B.F., Bleeg, I.S., Christensen, B.T., Kondo, T., Brandt, K., 2003. Flavone C-glycoside, phenolic acid, and nitrogen contents in leaves of barley subject to organic fertilization treatments. J. Agric. Food Chem. 51, 809–813.
Pan, H., Lundgren, Lennart N., 1994. Rhododendrol glycosides and phenyl glucoside esters from inner bark of *Betula pubescens*. Phytochemistry 36, 79–83.
Seto, R., Nakamura, H., Nanjo, F., Hara, Y., 1997. Preparation of epimers of tea catechins by heat treatment. Biosci. Biotechnol. Biochem. 61, 1434–1439.
So, H.M., Yu, J.S., Khan, Z., Subedi, L., Ko, Y.J., Lee, I.K., Park, W.S., Chung, S.J., Ahn, M.J., Kim, S.Y., Kim, K.H., 2019. Chemical constituents of the root bark of *Ulmus davidiana* var. *japonica* and their potential biological activities. Bioorg. Chem. 19, 103145.
Souza, L.M.D., Cipriani, T.R., Iacomini, M., Gorin, P.A.J., Sassaki, G.L., 2008. HPLC/ESI-MS and NMR analysis of flavonoids and tannins in bioactive extract from leaves of *Maytenus ilicifolia*. J. Pharm. Biomed. Anal. 47, 59–67.
Wang, D., Xia, M.Y., Cui, Z., 2006. New triterpenoids isolated from the root bark of *Ulmus pumila* L. Chem. Pharm. Bull. 54, 775–778.
Wang, L.B., Gao, H.Y., Morikawa, T., Sun, B.H., Huang, J., Yoshikawa, M., Wu, L.J., 2009. Flavonones from *Helichrysi flos* syn. Chin. J. Nat. Med. 7, 357–360.
Yang, H., Son, J.K., Jung, B., Zheng, M.S., Kim, J.R., 2011. Epifriedelanol from the root bark of *Ulmus davidiana* inhibits cellular senescence in human primary cells. Planta Med. 77, 441–449.
You, Y.O., Choi, N.Y., Kim, K.J., 2013. Ethanol extract of *Ulmus pumila* root bark inhibits clinically isolated antibiotic-resistant bacteria. Evid. Based Complement Altern. Med. 2013, 269–274.
Zerbib, M., Mazauric, J.P., Meudec, E., Guernevé, C.L., Lepak, A., Nidetzky, B., Cheynier, V., Terrier, N., Saucier, C., 2018. New flavanol O-glycosides in grape and wine. Food Chem. 266, 441–448.
Zheng, M.S., Hosny, M., Zhang, H., Chang, H.W., Woo, M.H., Son, J.K., Lee, S.K.S., 2014. (-)-catechin glycosides from *Ulmus davidiana*. Arch Pharm. Res. (Seoul) 37, 698–705.
Zheng, M.S., Lee, Y.K., Li, Y., Hwangbo, K., Lee, C.S., Kim, J.R., Lee, S.K.S., Chang, H.W., Son, J.K., 2010a. Inhibition of DNA topoisomerases I and II and cytotoxicity of compounds from *Ulmus davidiana* var. japonica. Arch Pharm. Res. (Seoul) 33, 1307–1315.
Zheng, M.S., Li, G., Li, Y., Seo, C.S., Lee, Y.K., Jung, J.S., Song, D.K., Bae, H.B., Kwak, S.H., Chang, H.W., Kim, J.R., Son, J.K., 2011. Protective constituents against sepsis in mice from the root barks of *Ulmus davidiana* var. *japonica*. Arch Pharm. Res. (Seoul) 34, 1443–1450.
Zheng, M.S., Yang, J.H., Li, Y., Li, X., Chang, H.W., Son, J.K., 2010b. Anti-inflammatory activity of constituents isolated from *Ulmus davidiana* var. *japonica*. Biomolecules Ther. 18, 321–328.