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Phenolic Glycosides from *Cercidiphyllum japonicum* Leaves[1]

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ABSTRACT

Cercidiphyllum japonicum leaves were 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. A portion of EtOAc fraction (10 g) was chromatographed on a Sephadex LH-20 column, by the successively elution with various aqueous MeOH-H_2O (1:9, fraction 1-2 → 3:7, fraction 3-5 → 1:1, fraction 6-9 → 7:3, fraction 10-13 → 9:1, fraction 14-16). Compound **2** was isolated from fraction 6 and compound **1** was separated from fraction 11 and 12. Compound **3** and **4** were purified from fraction 13. The isolated compounds were elucidated as quercetin-3-*O*-α-L-rhamnopyranoside **(1)**, chlorogenic acid **(2)**, quercetin-3-*O*-α-L-arabinofuranoside **(3)** and quercetin-3-*O*-β-D-xylopyranoside **(4)** by the spectral and literature data, and by comparison with the authentic samples. These compounds were reported, for the first time, from the extracts of *C. japonicum* leaves. Also chlorogenic acid **(2)** has never been reported before in domestic tree species and can be used as an index compound for *C. japonicum*.

Keywords : *Cercidiphyllum japonicum* leaves, phenolic glycosides, ethyl acetate fraction, column chromatography, structure elucidation

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

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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 al*., 2005). Because of its extremely low ability of regeneration in natural population, the number of its populations is very little. Therefore, the tree is now treated as "endangered" in China and recognized globally as lower risk under the International Union for the Conservation of Nature criteria.

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 a hydrolyzable tannin is one of those sources.

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 functional uses in domestic or abroad (Towatari *et al*., 2002; Tada and Sakurai, 1991; Takasugi and Katui, 1986).

This work was carried out, for the first time in domestic, to investigate the chemical constituents of the extracts of katsura tree leaves for future functional use, and to elucidate the structures of phenolic glycosides from the leaf extracts isolated by column chromatography analysis.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh *Cercidiphyllum japonicum* leaves were collected at Hwacheon, Gangwon-do in August 2013, air dried for two weeks and then ground to fine particles to be extracted.

2.2. Sample preparation

The ground leaves (3 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 rotary evaporator under the reduced pressure at 40℃. The aqueous crude residue was successively fractionated on a separatory funnel and freeze dried to give *n*-hexane (2.6 g), CH_2Cl_2 (8.8 g), EtOAc (35.2 g), and H_2O (45.2 g) soluble fractions.

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 δ (ppm).

FAB-MS and MALDI-TOF-MS were performed with a Micromass Autospec M363

spectrometer.

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) and by spraying vanillin (Vanillin-EtOH-H_2SO_4 (15:250:2.5, w/v/v)), then heating.

2.5. Column chromatography

A portion of EtOAc fraction (10 g) was chromatographed on a Sephadex LH-20 column, successively eluting with MeOH-H_2O (1:9, fraction 1-2 → 3:7, fraction 3-5 → 1:1, fraction 6-9 → 7:3, fraction 10-13 → 9:1, fraction 14-16). Compound **2** was isolated from fraction 6 and compound **1** was isolated from fraction 11 and 12. Compound **3** and **4** were separated from fraction 13.

2.5.1. Quercetin-3-*O*-*α*-L-rhamnopyranoside (1)

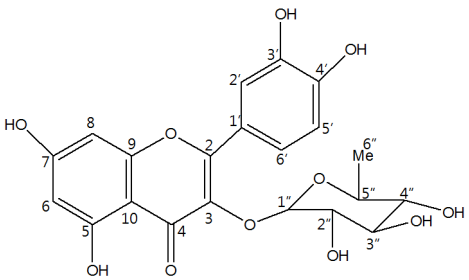
Yellow amorphous powder. R_f : 0.58 (TBAW) and 0.25 (6% HOAc). MALDI-TOF-MS : m/z 471 $[M+Na]^+$, 449 $[M+H]^+$. ^{1}H-NMR (CD_3OD, δ) : 0.95 (3H, *d*, *J* = 6.14 *Hz*, rham H-6), 3.42 (1H, *m*, rham H-5), 3.66 (1H, *m*, rham H-4), 3.76 (1H, *dd*, *J* = 3.40 *Hz*, 3.23, rham H-3), 4.23 (1H, *dd*, *J* = 1.63 *Hz*, 1.66, rham H-2), 5.36 (1H, *d*, *J* = 1.5 *Hz*, rham H-1), 6.20 (1H, *d*, *J* = 2.0 *Hz*, H-6), 6.36 (1H, *d*, *J* = 2.0 *Hz*, H-8), 6.91 (1H, *d*, *J* = 8.5 *Hz*, H-5'), 7.31 (1H, *dd*, *J* = 2.2 *Hz* and *J* = 8.5 *Hz*, H-6'), 7.34 (1H, *d*, *J* = 2.2 *Hz*, H-2'). ^{13}C-NMR (CD_3OD, δ) : 17.69 (rham C-6), 71.93 (rham C-5), 72.06 (rham C-3), 72.13 (rham C-2), 73.28 (rham C-4), 94.74 (C-8), 99.83 (C-6), 103.56 (rham C-1), 105.92 (C-10), 116.39 (C-2'), 116.96 (C-5'), 122.92 (C-6'), 122.99 (C-1'), 136.26 (C-3), 146.42 (C-3'), 149.80 (C-4'), 158.52 (C-9), 159.32 (C-2), 163.21 (C-5), 165.87 (C-7), 179.65 (C-4).

2.5.2. Chlorogenic acid (2)

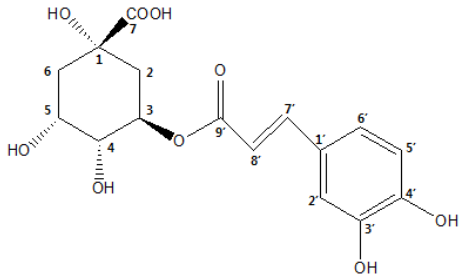
White greyish powder. R_f : 0.58 (TBAW) and 0.24 (6% HOAc). FAB-MS : m/z 367 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, δ) : 1.98～2.26 (4H, *m*, H-2, 6), 3.71 (1H, *m*, H-4), 4.15 (1H, *m*, H-5), 5.32 (1H, *m*, H-3), 6.15 (1H, *d*, *J* = 15.9 *Hz*, H-8'), 6.77 (1H, *d*, *J* = 8.2 *Hz*, H-5'), 6.94 (1H, *dd*, *J* = 2.0 and 8.2 *Hz*, H-6'), 7.03 (1H, *d*, *J* = 2.0 *Hz*, H-2'), 7.42 (1H, *d*, *J* = 15.9 *Hz*, H-7'). ^{13}C NMR (DMSO-d_6, ppm): 36.2 (C-6), 37.2 (C-2), 68.0 (C-5), 70.3 (C-4), 70.9 (C-3), 73.5 (C-1), 114.3 (C-8'), 114.7 (C-2'), 115.7 (C-5'), 121.2 (C-6'), 125.6 (C-1'), 144.8 (C-7'), 145.5 (C-3'), 148.3 (C-4'), 165.7 (C-9'), 175.0 (C-7').

2.5.3. Quercetin-3-*O*-*α*-L-arabinofuranoside (3)

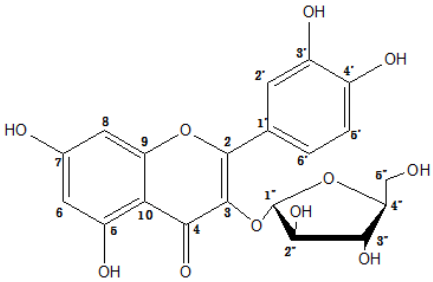
Yellow orange powder. R_f : 0.79 (TBAW) and 0.16 (6% HOAc). FAB-MS : m/z 457 $[M+Na]^+$. ^{1}H NMR (CD_3OD, δ): 3.42 (2H, *m*, Ara H-5), 3.77 (1H, *m*, Ara H-3), 3.82 (1H, *m*, Ara H-2), 4.23 (1H, *dd*, Ara H-4), 5.48 (1H, *s*, Ara H-1), 6.24 (1H, *d*, *J* = 1.5 *Hz*, H-6), 6.42 (1H, *d*, *J* = 1.5 *Hz*, H-8), 6.93 (1H, *d*, *J* = 7.9 *Hz*, H-5'), 7.52 (1H, *dd*, *J* = 2.1 and 7.9 *Hz*, H-6'), 7.56 (1H, *d*, *J* = 2.1 *Hz*, H-2'). ^{13}C NMR (CD_3OD,



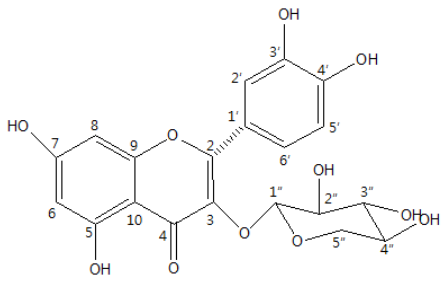
quercetin-3-*O*-α-L-rhamnopyranoside **(1)**



chlorogenic acid **(2)**



quercetin-3-*O*-α-L-arabinofuranoside **(3)**



quercetin-3-*O*-β-D-xylopyranoside **(4)**

Fig. 1. Structures of the isolated compounds.

ppm): 61.12 (Ara C-5), 77.28 (Ara C-3), 81.90 (Ara C-2), 86.62 (Ara C-4), 93.36 (C-8), 98.48 (C-6), 104.22 (C-10), 108.12 (Ara C-1), 114.58 (C-2'), 115.42 (C-5'). 130.59 (C-1'), 121.56 (C-6'), 133.51 (C-3), 144.65 (C-3'), 148.45 (C-4'), 157.02 (C-9), 157.52 (C-2), 161.66 (C-5), 164.60 (C-7), 177.99 (C-4).

2.5.4. Quercetin-3-*O*-β-D-xylopyranoside (4)

Yellow amorphous powder. R_f : 0.76 (TBAW) and 0.14 (6% HOAc). FAB-MS : m/z 457 $[M+Na]^+$. ^1H NMR (CD_3OD, δ) : 3.0 (1H, *dd*, *J* = 9.42 and 9.60 *Hz*, Xyl H-5), 3.30 (1H, *m*, Xyl H-4), 3.32 (1H, m, Xyl H-3), 3.43 (1H, *m*, Xyl H-2), 3.68 (1H, *dd*, *J* = 4.08 and 5.13 *Hz*, Xyl H-5), 5.1 (1H, *d*, *J* = 7.52 *Hz*, Xyl H-1), 6.1 (1H, *d*, *J* = 1.5 *Hz*, H-6), 6.3 (1H, *d*, *J* = 1.5 *Hz*, H-8), 6.80 (1H, *d*, *J* = 2.1 *Hz*, H-2'), 7.57 (1H, *d*, *J* = 7.9 *Hz*, H-5'), 7.47 (1H, *dd*, *J* = 2.1 and 7.9 *Hz*, H-6'). ^{13}C NMR (CD3OD, ppm) : 63.81 (Xyl C-5), 69.59 (Xyl C-3), 73.86 (Xyl C-2), 76.24 (Xyl C-4), 93.35 (C-8), 98.47 (C-6), 103.20 (Xyl C-1), 104.24 (C-10), 115.03 (C-2'), 115.80 (C-5'). 121.89 (C-6'), 130.77 (C-1'), 134.0 (C-3), 144.96 (C-3'), 148.48 (C-4'), 157.17 (C-9), 157.95 (C-2), 161.68 (C-5), 164.63 (C-7), 178.59 (C-4).

3. RESULTS AND DISCUSSION

The compounds were isolated from the EtOAc fraction of the extracts of katsura tree (*Cerdidiphyllum japonicum* Sieb, Et Zucc)

leaves 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. Quercetin-3-*O*-α-L-rhamnopyranoside (1)

Compound **1** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (7:3, v/v) eluent.

The ^{1}H NMR spectrum of aglycon gave three proton signals at δ 7.34 (1H, *d*, *J* = 2.1 *Hz*), δ 7.31 (1H, *dd*, *J* = 2.1 and 8.5 *Hz*), and δ 6.91 (1H, *d*, *J* = 8.5 *Hz*) attributable to H-2', H-6', and H-5', respectively. Also two *meta*-coupled doublets at δ 6.20 and δ 6.36 (2H, *J* = 2.0 *Hz*) identical to H-6 and H-8, respectively.

In the ^{13}C NMR spectrum, six oxygen-containing aromatic carbons appeared at 136.26 ppm (C-3), 163.21 ppm (C-5), 165.87 ppm (C-7), 158.52 ppm (C-9), 146.42 ppm (C-3'), and 149.80 ppm (C-4'). One carbonyl gave a signal at 179.65 ppm (C-4).

According to the above spectral and literature data, the aglycon was identified as quercetin (Kwon *et al*., 2007; Luo *et al*., 2009).

In the ^{1}H NMR spectrum of sugar moiety, an anomeric proton signal at δ 5.36 (1H, *d*, *J* = 1.5 *Hz*, H-1") and a methyl proton signal at δ 0.95 (3H, *d*, *J* = 6.14 *Hz*, H-6") were observed, which are characteristic of α-L-rhamnopyranoside.

In the ^{13}C NMR spectrum, typical rhamnose signals were indicated at 103.56 ppm (C-1"), 72.13 ppm (C-2"), 72.06 ppm (C-3"), 73.28 ppm (C-4"), 71.93 ppm (C-5"), and 17.69 ppm (C-6") (Agrawal, 1989).

On the basis of the above spectral data and by comparison of the literature data (pyo *et al*., 2002; Lee *et al*., 2004), compound **1** was elucidated as quercetin-3-*O*-α-L-rhamnopyranoside, quercitrin.

3.2. Chlorogenic acid (2)

Compound **2** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (1:1, v/v) eluent.

In the ^{1}H NMR spectrum of the caffeoyl moiety, three aromatic proton signals were indicated at 7.03 (1H, *d*, *J* = 2.0 *Hz*), 6.94 (1H, *dd*, *J* = 2.0 and 8.2 *Hz*), and 6.77 (1H, *d*, *J* = 8.2 *Hz*) attributable to H-2', H-6', and H-5', respectively. Also two doublet signals at δ 6.15 and δ 7.42 with the coupling constant of 15.9 *Hz* were attributed to the *trans* olefinic protons, H-8' and H-7', respectively.

In the ^{13}C NMR spectrum, the carboxyl acid carbon (C-9') was appeared at 174.9 ppm and two trans olefinic carbons, C-8' and C-7', were resonated at 114.3 ppm and 145.5 ppm, respectively.

According to the above spectral and authentic data, this aglycon was identified as caffeic acid (Cheminar *et al*., 1988; Dey and Harborne, 1989; Kim *et al*., 2002).

In the ^{1}H NMR spectrum of the quinic acid moiety, H-2 and H-6 gave multiplet signals for four protons at δ 1.98-δ 2.26. Also H-3 of the carbon linked to the caffeoyl group was ap-

peared downfield at δ 5.32 as a sextet signal due to the conjugation effect with the carbonyl group of the caffeoyl. H-4 and H-5 which are attached to the other hydroxyl containing carbons were resonated at δ 3.71 and δ 4.15, respectively. These proton chemical shifts were identical to the literature (Hyun *et al*., 2010).

In the ^{13}C NMR spectrum of the quinic acid moiety, two methylene carbons, C-2 and C-6, appeared at 37.2 ppm and 36.2 ppm, respectively. C-2 was shifted upfield about 4 ppm due to the steric effect from the caffeoyl moiety. C-4 which is linked to the caffeoyl moiety gave a downfield signal at 70.3 ppm due to the conjugation effect from the caffeoyl group. The other hydroxyl containing carbons, C-3 and C-5, appeared at 70.9 ppm and 68.0 ppm, respectively. The quarternary carbon, C-1, was resonated at 73.5 ppm and the carboxylic acid carbon, C-7, gave a signal at 175.0 ppm.

These ^{13}C NMR values were also similar to those of the literature (Hyun *et al*., 2010).

On the basis of the above spectral data and by comparison of the literature data, compound **2** was elucidated as chlorogenic acid.

3.3. Quercetin-3-*O*-α-L-arabinofuranoside (3)

Compound **3** was isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (7:3, v/v) eluent.

^{1}H and the ^{13}C NMR spectra of the aglycon were very much similar to those of compound **1** which was elucidated as quercetin.

In the ^{1}H NMR spectrum of the sugar moiety, two methylene protons gave a multiplet signal at δ 3.42 for H-5.

H-2 and H-3 also gave two multiplet signals at δ 3.82 and δ 3.77, respectively. H-4 indicated a double doublet signal at δ 4.23 and anomeric H-1 gave a typical singlet signal at δ 5.48.

In the ^{13}C NMR spectrum of the sugar moiety of compound **3**, the oxygen-containing C-1 was resonated at 108.12 ppm due to the conjugation with C-3 of quercetin. The other carbons, C-2, C-3, C-4 and C-5, gave signals at 81.90 ppm, 77.28 ppm, 86.62 ppm and 61.12 ppm, respectively.

HMBC spectrum also indicated a correlation between C-3 (133.51 ppm) of quercetin and H-1 (δ 5.48) of arabinose.

On the basis of the above spectral data and by comparison of the literature data (Kim *et al*., 1997; Han *et al*., 2004; Agrawal, 1989; Guo *et al*., 1998; Markham, 1992; Harborne and Mabry, 1982; Dawidar *et al*., 2014), compound **3** was elucidated as quercetin-3-*O*-α-L-arabinofuranoside, avicularin.

3.4. Quercetin-3-*O*-β-D-xylopyranoside (4)

Compound **4** was also isolated from Sephadex LH-20 column chromatography using MeOH-H_2O (7:3, v/v) eluent.

^{1}H and the ^{13}C NMR spectra of the aglycon of compound **4** were also very much similar to those of compound **1** and **3**, and the aglycon was elucidated as quercetin.

In the ^{1}H NMR spectrum of the sugar moiety, two methylene protons gave two signals at δ 3.0 and δ 3.68 for H-5.

H-2 and H-3 also gave two signals at δ 3.43 and δ 3.32, respectively. H-4 indicated a double doublet signal at δ 3.3 and anomeric H-1 gave a typical doublet signal at δ 5.1 with β coupling constant of 7.25 *Hz*.

In the ^{13}C NMR spectrum of the sugar moiety, the oxygen-containing C-1 was resonated at 103.20 ppm due to the conjugation with C-3 of quercetin. The other carbons, C-2, C-3, C-4 and C-5, gave signals at 73.86 ppm, 69.59 ppm, 76.24 ppm and 63.81 ppm, respectively.

HMBC spectrum also indicated a correlation between C-3 (134.0 ppm) of quercetin and H-1 (δ 5.10) of xylose.

On the basis of the above NMR data and by comparison of the literature data (Agrawal, 1989; Bergeron *et al*., 1997; Dubeler *et al*., 1996), compound **4** was elucidated as quercetin-3-*O*-β-D-xylopyranoside.

4. CONCLUSION

Katsura tree (*Cercidiphyllum japonicum* Sieb. Et Zucc) leaves 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 EtOAc fraction (10 g) was chromatographed on a Sephadex LH-20 column, by the successively elution with various aqueous MeOH-H_2O (1:9, fraction 1-2 → 3:7, fraction 3-5 → 1:1, fraction 6-9 → 7:3, fraction 10-13 → 9:1, fraction 14-16).

Compound **2** was isolated from fraction 6 and compound **1** was separated from fraction 11 and 12. Compound **3** and **4** were purified from fraction 13.

The isolated compounds were elucidated as quercetin-3-*O*-α-L-rhamnopyranoside **(1)**, chlorogenic acid **(2)**, quercetin-3-*O*-α-L-arabinofuranoside **(3)** and quercetin-3-*O*-β-D-xylopyranoside **(4)** by the spectral and literature data, and by comparison with the authentic samples.

These compounds were reported, for the first time, from the extracts of *C. japonicum* leaves.

Especially, chlorogenic acid **(2)** has never been reported before in domestic tree species and can be used as an index compound for *C. japonicum*.

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