

Chemotaxonomic Significance of Catechin 7-O-beta-D-apiofuranoside in *Ulmus* Species¹

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ABSTRACT

Ulmus genus has excellent various physiological activities, including anti-ulcer, antioxidant, antibacterial, anti-cancer, immunity, and homeostasis maintenance effects, and it is known to have many additional drug effects. And one of reasons for these excellent biological activities is a flavan-3-ol chemical group in *Ulmus* genus. In this study a new flavan-3-ol compound was identified in *Ulmus davidiana* var. *japonica*. A flavan-3-ol, (2R,3S)-7-[(2S,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)oxolan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5-diol, named as catechin 7-O-beta-D-apiofuranoside, was isolated from the stems and barks of *Ulmus davidiana* var. *japonica* for. *suberosa*, which is a species belonging to the genus *Ulmus*, growing throughout the Korea peninsula. The structure was elucidated by various spectroscopic methods including high-resolution TOF mass spectrometry, ¹H-NMR and ¹³C-NMR spectrometry and comparison with chemical structures of defined compounds.

Keywords: *Ulmus davidiana* var. *japonica* for. *suberosa*, flavan-3-ol, chemotaxonomy

1. INTRODUCTION

More than 30 species belonging to *Ulmus* genus grow in temperate regions of the Northern Hemisphere. Six species and two varieties of this genus are found in Korea: *U. davidiana* Planchon, *U. davidiana* Planchon var. *japonica* Nakai, *U. laciniata* Mayr, *U. macrocarpa* Hence, *U. macrophylla* Nakai, *U. parvifolia* Jacq. (*U. coreana* Nakai), *U. pumila* Linne (*U. mandshurica* Nakai) and *Ulmus davidiana* var. *japonica* for. *Suberosa* (Jung, 1974; Lee, 1996).

Flavan-3-ols are characteristic chemical components of *Ulmus* species (Son *et al.*, 1989; Moon *et al.*, 1995; Doskotch *et al.*, 1973; Jung *et al.*, 2007). Several interesting biological activities of flavan-3-ols including anti-inflammatory, anti-cancer, analgesic, antimicrobial, antineoplastic, anti-ulcer, antioxidant, antibacterial, anticancer and immunomodulatory properties have been reported previously (Hong *et al.*, 1990; Park *et al.*, 1996; Yang *et al.*, 1996; Cho *et al.*, 1996; Jun *et al.*, 1998; Lee *et al.*, 2000; Lee *et al.*, 2001; Kim *et al.*, 2002).

A flavan-3-ol compound, (+)-catechin 7-O-β-D-xy-

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lopyranoside was isolated from the bark of *U. Americana* (Doskotch *et al.*, 1973). In addition, (+)-catechin and (+)-catechin 5-O- β -D-apiofuranoside from roots of *U. davidiana* were isolated (Son *et al.*, 1989). Isoquercetin and rutin from leaves of *U. parvifolia* were isolated (Kim *et al.*, 1992). Sterols, sterol glucoside and (+)-catechin 7-O- α -L-rhamnoside from barks of *U. parvifolia* were isolated (Moon *et al.*, 1995). Sesquiterpene O-naphthoquinone of davidianone A, B, C and mansonone E, F, H, I from roots of *U. davidiana* were isolated (Kim *et al.*, 1996). (+)-Catechin 7-O- β -D-apiofuranoside, lyoniside, 5'-methoxyisolariciresinol-9'-O- β -D-xylopyranoside, rel-trans dihydrodehydroconiferyl alcohol 4-O- α -L-rhamnoside, icariside and ulmicine A-E from barks and roots of *U. davidiana* var. *japonica* were isolated (Bae *et al.*, 2000), respectively.

In this study, we isolated and identified catechin 7-O-beta-D apiofuranoside from the stems and barks of *U. davidiana* var. *japonica* for. *suberosa*. We also report the screening of catechin 7-O-beta-D apiofuranoside from other *Ulmus* species [*U. davidiana* Planchon (leaves, stems and barks), *U. davidiana* var. *japonica* (leaves, stems), *U. parvifolia* Jacq (stems and barks) and *U. pumila* Linne (stems)].

2. MATERIALS and METHODS

2.1. Plant extracts of *Ulmus* species

The stems and barks of *U. davidiana* var. *japonica* for. *suberosa* (bar code; PB2437.1) as well as MeOH extracts of *U. pumila* Linne (stems, bar code; PB2430.2), *U. parvifolia* Jacq (stems and barks, bar code; PB2427.1), *U. davidiana* var. *japonica* (stems, leaves, bar code; PB2436.6~7), and *U. davidiana* Planchon (leaves, stems and barks, bar code; PB2435.1~2) were purchased from the Korea Plant Extract Bank(Cheongju, Korea).

2.2. Chemical separation and identification

Thin layer chromatography (TLC) was carried out using a pre-coated silica gel 60 F₂₅₄ plate (Merck, Darmstadt, Germany) with chloroform, methanol and water (70:30:4, volume ratio). The spots were detected under UV radiation (254 nm) and by spraying with FeCl₃ and 10% H₂SO₄ followed by heating.

The components derived from the *Ulmus* species were identified by several instrumental analyses. Based on 1D nuclear magnetic resonance (NMR), ¹H-(400MHz) and ¹³C-(100MHz) NMR experiments were recorded with FT-NMR Spectrometer 400 MHz, AVANCE III HD 400 (Bruker Corporation, Billerica, Massachusetts, USA) at Chonnam National University. Also, a high-resolution TOF mass spectrometry was conducted with JMS-T200GC (JEOL, Tokyo, Japan) at the National Center for Inter-University Research Facilities on Chonnam National University.

2.3. Quantitative analysis of catechin

7-O- β -D apiofuranoside of *Ulmus* species using high pressure liquid chromatography (HPLC)

HPLC was used for the quantitative analysis of catechin 7-O-beta-D apiofuranoside. A Waters 2695 separation module (Milford, MA, USA) equipped with a vacuum degasser, a binary pump, a 2487 dual λ absorbance detector and column compartment, was used to separate 1 on a Phenomenex KJ0-4282 guard column and SkyPakC18 column (4.6 \times 250 mm, 5 μ m particle) with a linear gradient [D.W. : MeOH : P₂HO₄ (940 : 50 : 1)] : [MeOH : P₂HO₄ (990 : 1)] = 100 : 0 to 0 : 100 for 30 min. The column temperature was maintained at room temperature. The flow rate was 1.0 mL/min. The injection volume of each sample was 20 μ L. The system was monitored at 280 nm (λ_{max} of catechin 7-O-beta-D apiofuranoside) eluting at 14.44 \pm 0.14 min, and catechin 7-O- β -D apiofuranoside was

detected in the extracts of the stems and barks of *U. davidiana* var. *japonica* for: *suberosa*, as well as the extracts of *U. davidiana* Planchon (leaves, stems and barks), *U. davidiana* var. *japonica* (leaves, stems), *U. parvifolia* Jacq (stems and barks) and *U. pumila* Linne (stems).

3. RESULTS and DISCUSSION

Brown amorphous powder, high-resolution TOF MS m/z : 422.17511 [M]⁺; ¹H-NMR (400 MHz, DMSO-*d*₆+D₂O): 6.74 (H-2', 1H, d, *J* = 2.0 Hz), 6.69 (H-5', 1H, d, *J* = 8.4 Hz), 6.59 (H-6', 1H, dd *J* = 2.0, 8.4 Hz), 6.09 (H-8, 1H, d, *J* = 2.4 Hz), 5.90 (H-6, 1H, *J* = 2.4 Hz), 5.33 (H-1'', 1H, d, *J* = 4.0 Hz), 4.55 (H-2, 1H, d, *J* = 7.2Hz), 4.03 (H-2'', 1H, d, *J* = 4.0 Hz), 4.00 (H-4a'', 1H, d, *J* = 9.6Hz), 3.89 (H-3, 1H, m), 3.68 (H-4b'', 1H, d, *J* = 9.6 Hz), 3.45 (H-5'', 2H, m), 2.65 (H-4a, 1H, dd, *J* = 4.8, 16.0Hz), 2.40 (H-4b, 1H, dd, *J* = 8.0, 16.0z); ¹³C-NMR (100 MHz, DMSO-*d*₆+D₂O): δ 156.7 (C-7), 156.5 (C-5), 155.7 (C-9), 145.2 (C-4'), 145.1 (C-3'), 130.8 (C-1'), 118.8 (C-6'), 115.6 (C-5'), 114.7 (C-2'), 107.3 (C-1''), 102.1 (C-10), 96.0 (C-8), 95.3 (C-6), 81.4 (C-2), 78.9 (C-3''), 76.3 (C-2''), 74.3 (C-4''), 66.3 (C-3), 62.4 (C-5''), 27.8 (C-4).

Compound catechin 7-O-beta-D apiofuranoside was brown amorphous powder. The spot was detected under UV radiation at 254 nm, the black spot was detected by spraying with FeCl₃ and dark brown and orange spots was detected by spraying 10 % H₂SO₄ and anisaldehyde- H₂SO₄ followed by heating, respectively.

An aromatic ABX-spin system, the presence of *meta*-coupled aromatic signal [δ 6.74 (1H, d, *J*=2.0 Hz, H-2'), *ortho*-coupled aromatic signal [δ 6.69 (1H, d, *J*=8.4 Hz, H-5')] and *ortho-meta*-coupled aromatic signal [δ 6.59 (1H, dd, *J*=2.0, 8.4 Hz, H-6')] was showed in ¹H-NMR spectrum. The two carbon signals of C-3' and 4' were observed in downfield (δ 145.1, 145.2) compared with the peaks of C-2', 5' and 6' (δ

114.7, 115.6 and 118.8) in ¹³C-NMR spectrum. These meant B-ring was pyrocatechol moiety which was substituted by hydroxyl group in C-3' and 4'. The signals of two more aromatic protons, which were *meta*-coupled ones [δ 5.90 (1H, d, *J*=2.4 Hz, H-6), δ 6.09 (1H, d, *J*=2.4 Hz, H-8)] were observed in ¹H-NMR spectrum, and the three carbon signals of C-5, C-7 and C-9, (δ 155.7, 156.5 and 156.7) were deshielded rather than C-6, 8 and 10 (δ 95.3, 96.0 and 102.1) in ¹³C-NMR spectrum.

These implied A-ring was related to phloroglucinol moiety which was substituted by hydroxyl group in C-5, 7 and 9. However the five carbon signals of 62.4 (api-5), 74.3 (api-4), 76.3 (api-2), 78.9 (api-3), 107.3 (api-1) indicate sugar existence in ¹³C-NMR spectrum. These signals were indicative of a D-apiofuranoside (Shengjun *et al.*, 2000). The linkage of this sugar at C-7 was established by comparing the spectral data with the values reported in the literature (Na *et al.*, 2002). Moreover, the proton signals of H-2 [δ 4.55 (1H, d, *J*=7.2 Hz)], H-3 [δ 3.89 (1H, m)], H-4_{eq} [δ 2.65 (1H, dd, *J*=4.8, 16.0 Hz)] and H-4_{ax} [δ 2.40 (1H, dd, *J*=8.0, 16.0Hz)] were observed in ¹H-NMR and the carbon signals in ¹³C-NMR spectrum of C-2, 3 and 4 (δ 81.4, 66.3 and 27.8) indicated flavan-3ol moiety. Especially, the large coupling constant of H-2 (*J*=7.2 Hz) in ¹H-NMR and carbon signals of C-2 at δ 81.4 in ¹³C-NMR suggested a 2,3-*trans*-configuration.

Thus, compound 1 was identified as catechin 7-O-β-D apiofuranoside based on the spectral data compared with the values reported in the previous studies (Jung *et al.*, 2007; Bae *et al.*, 2000; Shengjun *et al.*, 2000; Na *et al.*, 2002).

We quantified catechin 7-O-beta-D apiofuranoside from the stem and bark extracts of *U. davidiana* var. *japonica* for: *Suberosa* [172.35 ± 0.158 ppm (μg/mL), 17.24 ± 0.016%] (Fig. 3), the leaf extracts of *U. davidiana* Planchon [191.38 ± 0.219 ppm (μg/mL), 19.14 ± 0.022%] (Fig. 4), the stem and bark extracts of *U.*

dauidiana Planchon [177.84 ± 0.209 ppm ($\mu\text{g/mL}$), $17.78 \pm 0.021\%$] (Fig. 5), the leaf extract of *U. dauidiana* var. *japonica* [176.11 ± 0.155 ppm ($\mu\text{g/mL}$), $17.61 \pm 0.015\%$] (Fig. 6), the stem extract of *U. dauidiana* var. *japonica* [41.27 ± 0.191 ppm ($\mu\text{g/mL}$), $4.13 \pm 0.019\%$] (Fig. 7), the stem and bark extracts of *U. parvifolia* Jacq [150.88 ± 0.205 ppm ($\mu\text{g/mL}$), $15.09 \pm 0.020\%$] (Fig. 8) and the stem extract of *U. pumila* Linne [61.69 ± 0.178 ppm ($\mu\text{g/mL}$), $6.17 \pm 0.018\%$] (Fig. 9), using a calibration equation ($y = 6857.3x - 42331$; $R^2 = 0.9985$). According to the results of the content analysis experiment using HPLC, there

was no significant difference in the content of the indicator substance in the other samples except for the two samples (the stem extract of *U. dauidiana* var.

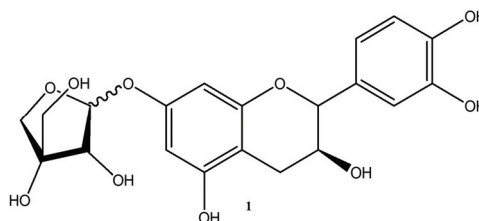


Fig. 1. Chemical structures of compound 1 isolated from *Ulmus* species.

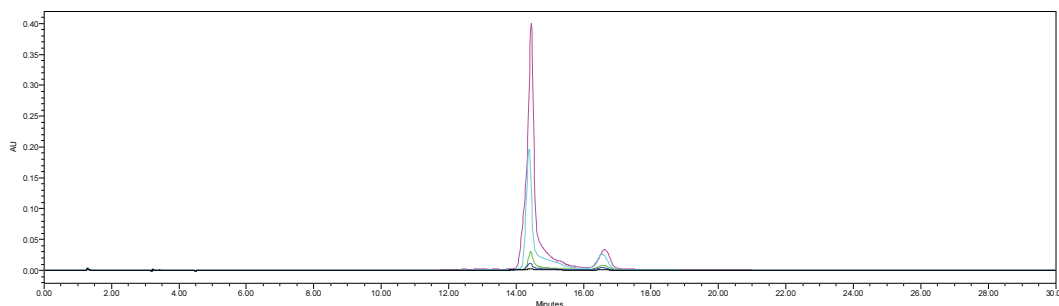


Fig. 2. HPLC chromatogram of compound 1. Sample 1 = Com. 1 1,000ppm(purple color line), Sample 2 = Com. 1 500 ppm (mint color line), Sample 3 = Com. 1 100 ppm (green color line), Sample 4 = Com. 1 50 ppm(blue color line).

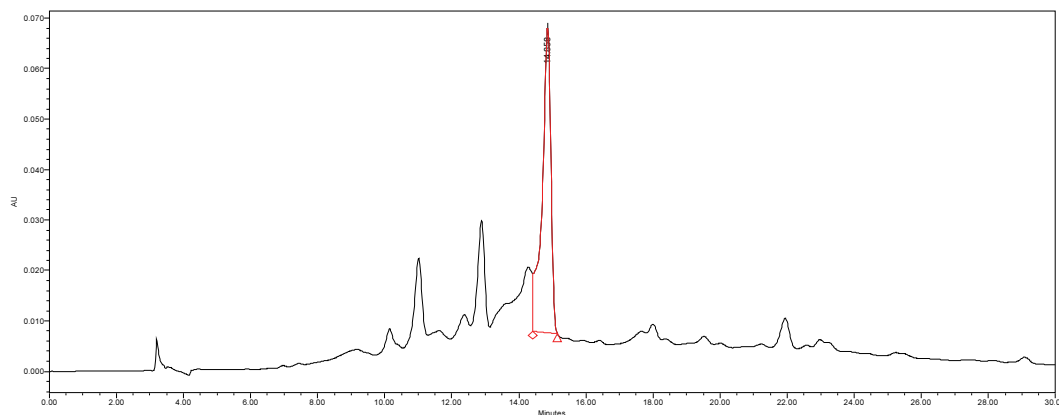


Fig. 3. HPLC chromatogram of the stem and bark extracts of *U. dauidiana* var. *japonica* for. *suberosa*. Sample 1 = stem and bark extracts of *U. dauidiana* var. *japonica* for. *suberosa*. 1,000 ppm.

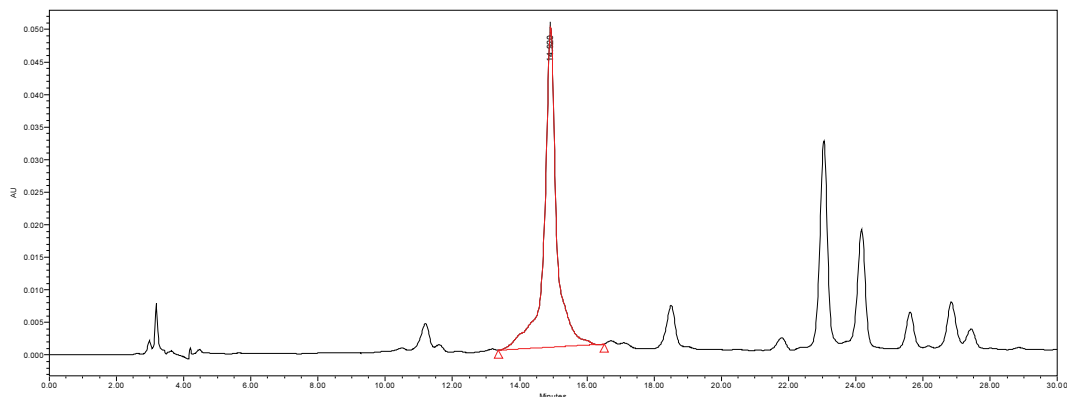


Fig. 4. HPLC chromatogram of the leaf extract of *U. davidiana Planchon*. Sample 1 = leaf extract of *U. davidiana Planchon*.1,000 ppm.

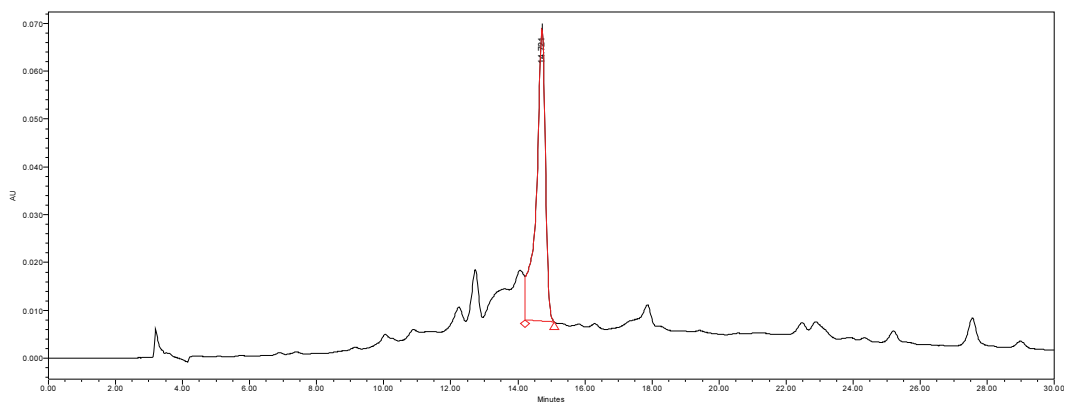


Fig. 5. HPLC chromatogram of the stem and bark extracts of *U. davidiana Planchon*. Sample 1 = stems and bark extract of *U. davidiana Planchon*.1,000 ppm.

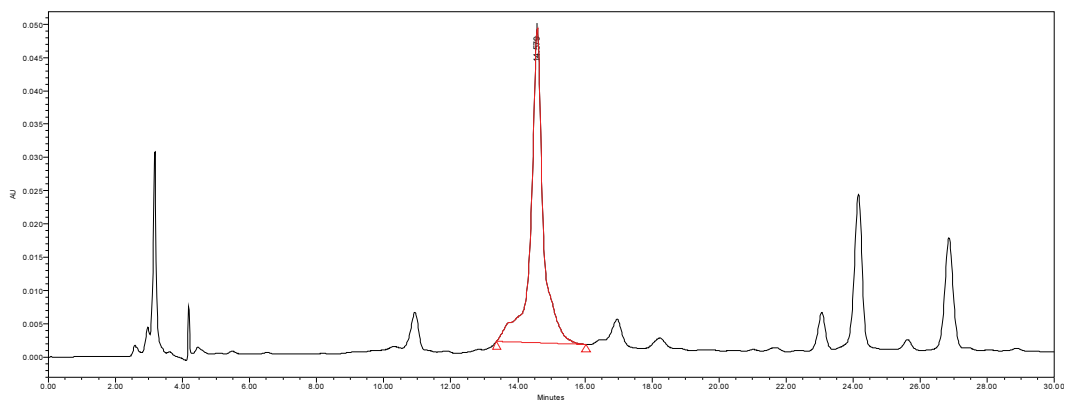


Fig. 6. HPLC chromatogram of the leaf extract of *U. davidiana var. japonica*. Sample 1 = leaf extract of *U. davidiana var. japonica*.1,000 ppm.

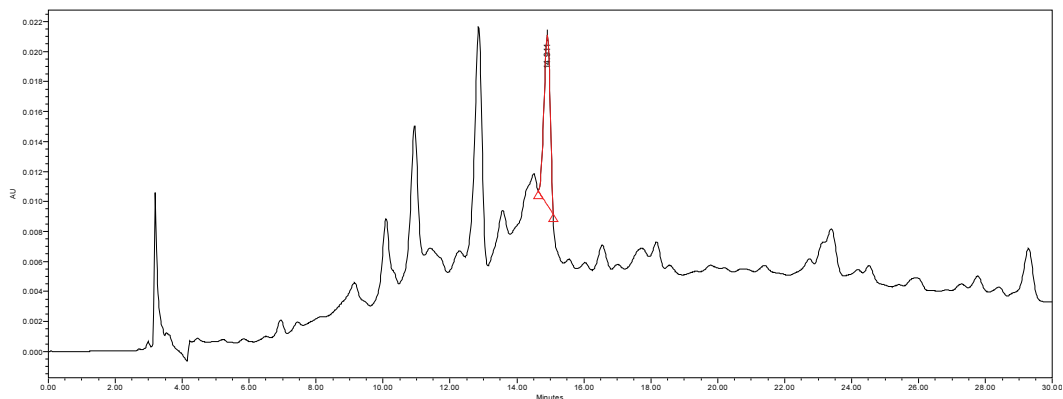


Fig. 7. HPLC chromatogram of the stem extract of *U. davidiana* var. *japonica*. Sample 1 = stem extract of *U. davidiana* var. *japonica*.1,000 ppm.

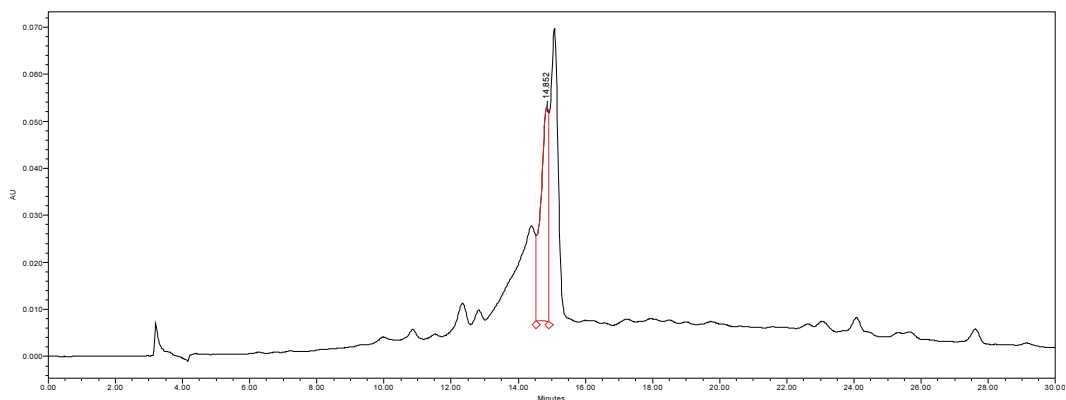


Fig. 8. HPLC chromatogram of the stem and bark extracts of *U. parvifolia* Jacq. Sample 1 = stems and bark extract of *U. parvifolia* Jacq.1,000 ppm.

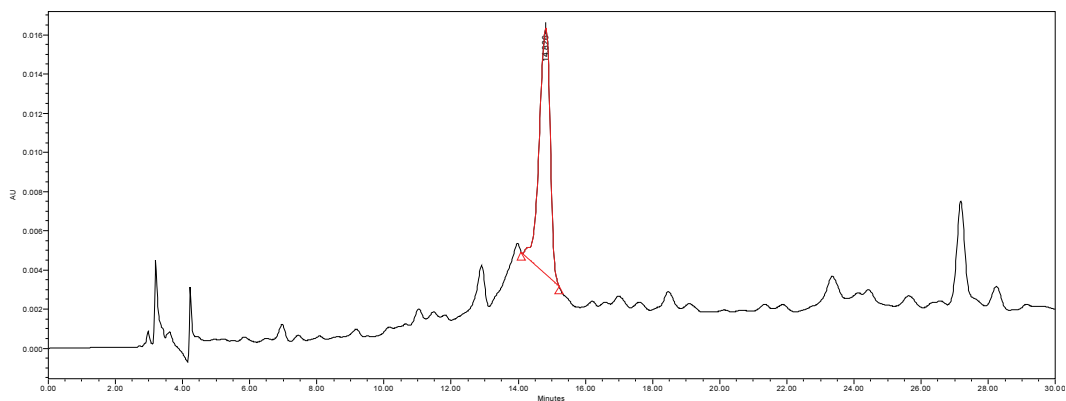


Fig. 9. HPLC chromatogram of the stem extract of *U. pumila* Linne. Sample 1 = stem extract of *U. pumila* Linne.1,000 ppm.

japonica and the stem extract of *U. pumila* Linne), but the two samples with a significantly lower level were important experimental results when evaluating the value of future use of raw materials. It is expected to be used as important basic data in future resource utilization.

4. CONCLUSION

This is the first report of the isolation and identification of catechin 7-O-beta-D apiofuranoside from *U. davidiana* var. *japonica* for. *suberosa*., *U. parvifolia* Jacq and *U. davidiana* Planchon. Since the initial isolation of catechin 7-O-beta-D apiofuranoside from *U. Americana* (Doskotch *et al.*, 1973), it has been found to be distributed among *U. davidiana* var. *japonica* (Jung *et al.*, 2007) and *U. macrocarpa* Hance (Kwon *et al.*, 2011).

Based on the above results, it is expected that it will be used as an important research result in searching for new natural materials that can be given high added value.

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