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Two new phenolic compounds from the leaves of *Alnus sibirica* Fisch. ex Turcz.

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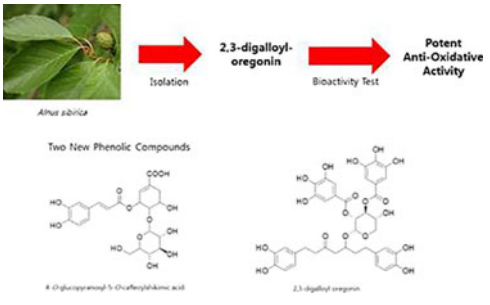

Two new phenolic compounds, 4-*O*-glucopyranosyl-5-*O*-caffeoylshikimic acid (**1**) and 2,3-digalloyl oregonin (**2**), were isolated along with eight known phenolic compounds (**3**–**10**) from an 80% acetone extract of *Alnus sibirica* leaves. The chemical structures of these compounds were elucidated using 1D/2D nuclear magnetic resonance and high resolution-MS. The anti-oxidative activities of these compounds were determined by assaying their 1,1-diphenyl-2-picrylhydrazyl radical and nitroblue tetrazolium superoxide anion scavenging activity. All of the isolated phenolic compounds (**1**–**10**) exhibited potent anti-oxidative activities. In particular, **2** and 4, which are diarylheptanoids, and **10** which is ellagitannin exhibited excellent anti-oxidative activities with almost the same potency as that of the positive controls *L*-ascorbic acid and allopurinol.

Keywords: *Alnus sibirica*; caffeoylshikimic acid; galloyl-diarylheptanoid; anti-oxidant activity

1. Introduction

Members of the *Alnus* species have been used in a number of traditional medicines such as cathartics, emetics, galactogogues, febrifuges, hemostatics, parasiticides, vermifuges, skin tonics and astringents (Guo et al. 2001). *Alnus sibirica* Fisch. ex Turcz. (AS) is geographically distributed in Korea, Japan, Northeast China and Russia, and the bark of this plant has been used as an antipyretic, expectorant, antiasthmatic and a health tea for alcoholism (Lee 1966). Previous studies on the chemical constituents of the *Alnus* species have led to the isolation of various

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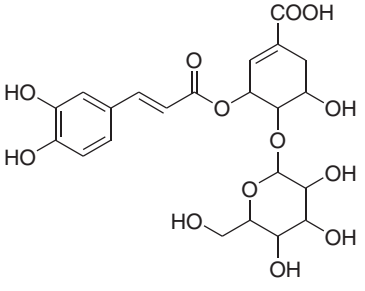
tannins, flavonoids, diarylheptanoids and triterpenoids (Suga et al. 1972; Terazawa et al. 1984; Aoki et al. 1990; Lee et al. 1992, 1999; Jeong et al. 2000; Choi et al. 2012). These studies have shown that the *Alnus* species is a good source of diarylheptanoids, and that plants of this genus exhibit anti-oxidative, anti-inflammatory, anti-atopic, anti-bacterial and anti-adipogenic activities (Joo et al. 2009; Lee et al. 2010, 2013; Choi et al. 2012). This paper describes the isolation and structure elucidation of two new phenolic compounds along with eight known phenolic compounds. In addition, the anti-oxidative activities of these compounds were determining by assessing their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and nitroblue tetrazolium (NBT) superoxide anion scavenging activity.

2. Results and discussion

The 80% acetone extract of AS leaves was dissolved in water and filtered using Celite. The resulting filtrate was concentrated and applied to column chromatography using Ambelite XAD-2, Sephadex LH-20, MCI-gel, CHP 20P and ODS-B gel with a reversed phase medium pressure liquid chromatography (MPLC) system, which afforded 10 compounds including two new phenolic compounds (**1** and **2**) (Figure 1). The known compounds (**3**–**10**) were identified as alnuside A (**3**, Kuroyanagi et al. 2005), alnuside C (**4**, Kuroyanagi et al. 2005), quercetin (**5**,



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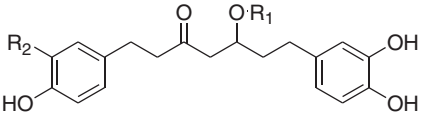
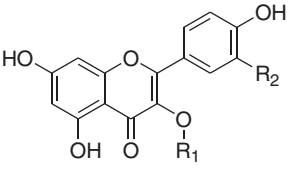


	R_1	R_2
2	(2,3-digalloyl)-xylose	OH
3	xylose	H
4	xylose-2-MeBu	OH



	R_1	R_2
5	H	OH
6	rhamnose	OH
7	galactose	OH
8	glucuronide-methyl ester	OH
9	coumaroyl rhamose	H

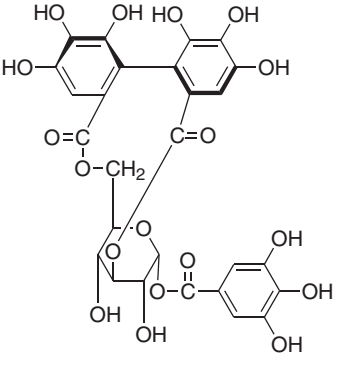


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Figure 1. Structures of compounds **1**–**10**.

Dutta et al. 2007), quercitrin (**6**, Lee et al. 2003), hyperoside (**7**, Lee et al. 2003), quercetin-3-*O*-β-D-glucuronide-methylester (**8**, Pacifico et al. 2013), kaempferol-3-*O*-α-L-(4″E-*p*-coumaroyl)-rhamnoside (**9**, Yang et al. 2010) and isocorilagin (**10**, Liu et al. 2008), respectively, by comparing their spectroscopic (MS and nuclear magnetic resonance (NMR)) data with literature values.

Compound **1** was isolated as a pale yellow amorphous powder. High-resolution (HR)-negative FAB-MS (*m/z* 497.1295 $[M - H]^-$, calcd for $C_{22}H_{25}O_{13}$, 497.1299) indicated its molecular formula was $C_{22}H_{26}O_{13}$. In thin-layer chromatography (TLC), **1** was detected using a UV lamp at 254 nm as a dark brown spot by spraying with $FeCl_3$ solution and a yellow spot by spraying with H_2SO_4 solution and heating.

The ^{1}H NMR spectrum of **1** revealed three aromatic protons [δ_H 7.18 (d, $J = 2.4$ Hz, H-2′), 7.03 (dd, $J = 2.4$, 7.8 Hz, H-6′), 6.84 (d, $J = 7.8$ Hz, H-5′)] in an ABX spin system and two doublets at δ_H 7.70 (d, $J = 16.2$ Hz, H-7′), 6.33 (d, $J = 16.2$ Hz, H-8′) of a *trans*-double bond. The ^{13}C NMR spectrum of **1** exhibited one carboxyl group (δ_C 167.3) and two hydroxyl bearing aromatic carbons (δ_C 148.2 and 146.2). These findings suggested the presence of a caffeoyl moiety.

The ^{1}H NMR spectrum of **1** showed the presence of a proton singlet at δ_H 6.72 (1H, m, H-6), two coupled germinal protons at δ_H 2.79 (1H, dd, $J = 5.4$, 18.6 Hz, H-2a) and 2.30 (1H, dd, $J = 5.4$, 18.6 Hz, H-2b) and three oxymethine groups appearing at δ_H 5.83 (1H, brs, H-5), 4.24 (1H, dd, $J = 5.1$, 12.6 Hz, H-3) and 4.10 (1H, dd, $J = 5.1$, 12.6 Hz, H-4). ^{13}C NMR spectrum of **1** revealed the presence of a shikimic acid moiety [δ_C 167.7 (C-7), 133.4 (C-1), 132.5 (C-2), 78.6 (C-4), 68.2 (C-5), 66.3 (C-3), 31.2 (C-6)].

The ^{1}H and ^{13}C NMR spectra of **1** also revealed a glucopyranosyl moiety, one methylene [δ_H 3.85 (m, H-6″a), 3.65 (m, H-6″b)], and five additional methine groups [δ_H 4.50 (d, $J = 7.8$ Hz, H-1″), 3.45 (m, H-3″), 3.34 (m, H-4″, H-5″), 3.23 (m, H-2″)] and at δ_C 103.6 (C-1″), 76.8 (C-5″), 76.4 (C-3″), 73.6 (C-2″), 70.2 (C-4″), 61.5 (C-6″). In addition, the large coupling constant ($J = 7.8$ Hz) of H-1″ at δ_H 4.50 in the ^{1}H NMR spectrum of **1** suggested that the glucopyranoside was in a β-configuration.

The connectivities of caffeoyl and shikimic acid and glucose were elucidated by heteronuclear multiple bond coherence (HMBC) correlation signals. The HMBC spectrum of **1** showed a correlation between the H-1″ of glucose and C-4 of shikimic acid, and also showed the correlation between H-5 of shikimic acid and carbonyl C-9′ of caffeoyl moiety. Based on these results, compound **1** was elucidated as 4-*O*-β-D-glucopyranosyl-5-*O*-caffeoylshikimic acid. Compound **1** is the first reported shikimic acid conjugated with glucose and caffeic acid.

Compound **2** was isolated as a dark yellow amorphous powder. HR-negative FAB-MS (*m/z* 781.1978 $[M - H]^-$, calcd for $C_{38}H_{37}O_{18}$, 781.1980) indicated its molecular formula was $C_{38}H_{38}O_{18}$. In TLC, **2** was detected as a dark blue spot by spraying with $FeCl_3$ solution, and a brown spot by spraying with H_2SO_4 solution and heating.

The ^{1}H and ^{13}C NMR spectra of **2** showed two galloyl groups in the aromatic region [δ_H 7.04 (2H, s, galloyl-2, 6) and 7.06 (2H, s, galloyl-2′, 6′)] and [(δ_C 165.7 (C-7‴), 165.0 (C-7⁗), 145.0 (C-3‴, 3⁗, 5‴, 5⁗), 138.1 (C-4‴), 138.0 (C-4⁗), 120.4 (C-1‴), 120.3 (C-1⁗), 109.2 (C-6‴, 6⁗), 109.1 (C-2‴, 2⁗)] and one diarylheptanoid glycoside moiety compose of five methylenes [δ_H 1.70–1.75 (2H in total, m, H-6), 2.33–2.68 (8H, m, H-1, 2, 4, 6, 7] and a methine [δ_H 4.15 (1H, m, H-5) and 1.70–1.75 (2H in total, m, H-6)] and carbonyl carbon C-3 (δ_C 207.7), a secondary carbonyl carbon C-5 (δ_C 75.2), and five carbons [δ_C 28.6 (C-1), 44.8 (C-2), 47.6 (C-4), 37.3 (C-6) and 30.5 (C-7)] as heptanes moiety and two sets of caffeoyl groups [δ_H 6.67 (1H, d, $J = 7.8$ Hz, H-5′), 6.69 (1H, d, $J = 7.8$ Hz, H-5″, 6.59 (1H, d, $J = 2.4$ Hz, H-2′), 6.67 (1H, d, $J = 2.4$ Hz, H-2″), 6.39 (1H, dd, $J = 2.4$, 7.8 Hz, H-6′) and 6.48 (1H, dd, $J = 2.4$, 7.8 Hz, H-6″; δ_C 115.0 (C-2′), 115.1 (C-2″), 115.3 (C-5′, C-5″) and 119.4 (C-6′, C-6″), 144.6 (C-3′), 144.7 (C-3″), 143.0 (C-4″) and 142.9 (C-4′)] and a xylopyranosyl moiety [δ_H 5.32 (1H, m, H-3‴), 5.07 (1H

dd, $J = 7.8, 9.6$ Hz, H-2‴), 4.81 (1H, d, $J = 7.8$ Hz, H-1‴), 4.04 (1H, m, H-5‴a), 3.95 (1H, m, H-4‴), and 3.48 (1H, m, H-5′b), and δ_C 101.2 (C-1‴), 75.7 (C-3‴), 71.7 (C-2‴), 68.4 (C-4‴) and 65.7 (C-5‴)] were observed.

These results indicated that **2** consisted of a diarylheptanoid glycoside (oregonin) and two galloyl groups. The locations of the galloyl units were determined as the C-3‴ and C-2‴ of the xylopyranosyl moiety by a downfield shift of C-3‴ and C-2‴ at δ_C 75.7 (C-3‴) and 71.7 (C-2‴) together with an upfield shift of C-4‴ to δ_C 68.4 (C-4‴) compared with oregonin (Lee et al. 1992).

The connectivity of the diarylheptanoid with xylose and galloyl groups was further confirmed by HMBC correlations. Especially, the HMBC spectrum of **2** showed a correlation between the H-1‴ of xylose and C-5 of the diarylheptanoid moiety. The HMBC spectrum of **2** also revealed correlations between H-2‴ and H-3‴ of xylose and each C-7⁗, 7⁗′ of the galloly moiety. Based on these results, the structure of compound **2** was elucidated as 2,3-digalloyl oregonin. Interestingly, a diarylheptanoid-conjugated ellagitannin from the leaves of *Alnus hirsuta* var. *microphylla* was previously reported (Lee et al. 1992); however, compound **2** is the first report of a diarylheptanoid-conjugated gallotannin.

Most of the isolated phenolic compounds from the leaves of AS exhibited potent scavenging activities of DPPH radical and NBT superoxide anion (Table 1). The new compound (**2**) which is diarylheptanoid with galloyl moiety and isocorilagin (**10**) which is ellagitannin showed more strong anti-oxidative activity than *L*-ascorbic acid and almost same potency compared with allopurinol (Table 1).

In particular, the anti-oxidative activity of **2**, which contains a diarylheptanoid with two galloyl moieties, is more potent than that of **3** and **4** whose structures are similar to **2** (Table 1). This result indicates the importance of polyhydroxy groups in the structure for the anti-oxidative effect.

3. Experimental

3.1. *General experimental procedures*

Column chromatographic isolations were performed using Ambelite XAD-2 (20–50 μm, Fluka AG, Buchs, Switzerland), Sephadex LH-20 (10–25 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical, Tokyo, Japan),

Table 1. DPPH radical and superoxide anion scavenging activities of compounds **1**–**10**.

Compounds	DPPH radical scavenging activity (μM)	Superoxide anion scavenging activity (μM)
1	10.28 ± 0.13^{c}	41.38 ± 0.52^{l}
2	6.66 ± 0.08^{a}	8.57 ± 0.36^{d}
3	33.85 ± 0.16^{n}	23.87 ± 0.28^{j}
4	11.43 ± 0.06^{d}	9.04 ± 0.09^{e}
5	21.82 ± 0.34^{k}	61.37 ± 1.47^{p}
6	17.74 ± 0.08^{i}	18.45 ± 0.74^{i}
7	18.56 ± 0.43^{j}	42.96 ± 0.23^{m}
8	29.31 ± 0.85^{l}	71.06 ± 0.62^{q}
9	41.24 ± 0.99^{o}	$>100^{r}$
10	6.92 ± 0.25^{a}	8.26 ± 0.420^{c}
Vit-C	20.50 ± 0.71^{k}	–
Allopurinol	–	7.51 ± 0.53^{b}

Values represent the mean ± SD of three determinations. In the IC_{50} column, values that are not significantly different from one another are designated with the same letter, whereas values that are significantly different from one another ($p < 0.05$) are designated with different letters.

Toyopearl HW-40F (30–60 μm, Tosoh Corp., Tokyo, Japan) and ODS-B gel (40–60 μm, Daiso, Osaka, Japan). ODS-B gel was also used as a stationary phase for the middle pressure liquid chromatography (MPLC) system. TLC was carried out using a pre-coated silica gel 60 F_{254} plate (Merck, Darmastadt, Germany) with chloroform, methanol, and water (70:30:4, 80:20:2, volume ratio). Spots were detected under UV radiation (254 nm) and spraying with $FeCl_3$ and 10% H_2SO_4 or anisaldehyde–H_2SO_4 followed by heating. The chemical structures were elucidated by several instrumental analyses. 1D NMR such as ^1H (300 or 600 MHz) and ^{13}C (75 or 150 MHz) NMR, 2D NMR such as proton–proton correlation spectroscopy (^1H–^1H COSY), heteronuclear single quantum coherence, and HMBC experiments were recorded with Gemini 2000 and VNS instruments (Varian, Palo Alto, CA, USA) at the centre for research facilities of Chung-Ang University. HR fast atom bombardment mass spectra were recorded with JMS-600W and JMS-700 instruments (JEOL, Tokyo, Japan) at the National Center for Inter-University Research facilities at Seoul National University.

3.2. *Plant material*

AS leaves were collected from Mt. Guksabong in Dongjak-gu, Seoul, Korea in August 2010 and its identity was confirmed by Prof. M.W. Lee (Pharmacognosy Lab, Laboratory of Pharmacognosy and Natural Product Derived Medicine, College of Pharmacy, Chung-Ang University) and C.I. Lee (Kwang-Leung Korean National Arboretum in Pocheon, Korea). A voucher specimen (MR2010-08) has been deposited at the herbarium of the College of Pharmacy, Chung-Ang University.

3.3. *Extraction and isolation*

AS leaves (15 kg) were extracted with 80% acetone at room temperature. The resulting extract was concentrated by removing the acetone under vacuum, which afforded 1062 g of material. After acetone evaporation, water liquid was filtered through Celite 545 (Duksan Pure Chemicals Co. Ltd, Korea). The resulting filtrate (764 g) was applied to Ambelite XAD-2 (20–50 μm, 10 kg, 70 × 50 cm) and eluted using a graded H_2O, 50–100% MeOH solvent system yielding six fractions (AS-1 to AS-6). Repeated column chromatography of fraction AS-4 (225 g) using a Sephadex LH-20 column (25–100 μm, 2000 g, 10 × 120 cm, 0–100% MeOH in H_2O) yielded seven subfractions (AS-4-1 to AS-4-7). Fraction AS-4-3 (94 g) was applied to a Sephadex LH-20 column (25–100 μm, 2000 g, 10 × 120 cm, 0–100% MeOH in H_2O), which yielded eight subfractions (AS-4-3-1 to AS-4-3-8). Repeated column chromatography of fraction AS-4-3-2 using an ODS gel column (50 μm, 150 g, 3 × 50 cm, 0–100% MeOH in H_2O) and Toyopearl HW-40F (40 μm, 120 g, 3 × 40 cm) yielded [4-*O*-glucopyranosyl-5-*O*-caffeoylshikimic acid] (**1**, 370 mg). Fraction AS-4-3-4 (9.79 g) was applied to an MCI gel column (50 μm, 400 g, 3 × 50 cm, 0–100% MeOH in H_2O) and an ODS gel column (50 μm, 250 g, 3 × 50 cm, 10–100% MeOH in H_2O) to yield alnuside A (**3**, 80 mg). Subfraction AS-4-3-7 (3.78 g) was applied to an ODS gel column (50 μm, 250 g, 3 × 50 cm, 20–100% MeOH in H_2O), which yielded quercitrin (**6**, 300 mg) and hyperoside (**7**, 22 mg). Subfraction AS-4-4 was applied to an MCI gel column (50 μm, 400 g, 3 × 50 cm, 0–100% MeOH in H_2O), from which 13 subfractions (AS-4-4-1 to AS-4-4-13) were obtained. Subfraction AS-4-4-3 was sequentially applied to an ODS gel column (50 μm, 250 g, 3 × 50 cm, 0–100% MeOH in H_2O), of which subfraction AS-4-4-3-11 was applied to a Toyopearl HW-40F column (40 μm, 120 g, 3 × 40 cm, 0–100% MeOH in H_2O) to yield isocorilagin (**10**, 55 mg). Likewise, the subfraction AS-4-4-5 was applied to an ODS gel column (50 μm, 250 g, 3 × 50 cm, 0–100% MeOH in H_2O), which yielded quercetin-3-*O*-β-D-glucuronide-methylester (**8**, 62 mg). In addition, 2,3-digalloyl oregonin (**2**, 66 mg) was obtained from subfraction AS-4-4-8 by column chromatography with an ODS gel column (50 μm, 250 g,

3 × 50 cm, 0–100% MeOH in H_2O). Repeated column chromatography of fraction AS-5 (40.85 g) using a Sephadex LH-20 column (25–100 μm, 2000 g, 10 × 120 cm, 50–100% MeOH in H_2O), yielded seven subfractions (AS-5-1 to AS-5-7). The subfraction AS-5-2 (1.37 g) was applied to an MCI gel column (50 μm, 400 g, 3 × 50 cm, 40–100% MeOH in H_2O) and an ODS gel column (50 μm, 150 g, 3 × 50 cm, 20–100% MeOH in H_2O), which yielded alnuside C (**4**, 45 mg). Fraction AS-5-5 (0.97 g) was continuously applied to an ODS gel column (50 μm, 150 g, 3 × 50 cm, 50–100% MeOH in H_2O), which resulted in kaempferol-3-*O*-α-L-(4″E-*p*-coumaroyl)-rhamnoside (**9**, 800 mg). Lastly, quercetin (**5**, 125 mg) was obtained by recrystallisation of AS-5-6 (0.49 g).

3.3.1. *4-O-Glucopyranosyl-5-O-caffeoylshikimic acid (1)*

Pale yellow amorphous powder. $[\alpha]_D$: − 149.7° ($c = 0.01$, MeOH). IR (KBr) cm^{-1}: 3391, 1697, 1601, 1517. HR-negative FAB-MS *m/z*: 497.1295 $[M - H]^-$ (calcd $C_{22}H_{25}O_{13}$, 497.1299). ^{1}H NMR (600 MHz, acetone-d_6 + D_2O): δ 7.70 (1H, d, $J = 16.2$ Hz, H-7′), 7.18 (1H, d, $J = 2.4$ Hz, H-2′), 7.03 (1H, dd, $J = 2.4$, 7.8 Hz, H-6′), 6.84 (1H d, $J = 7.8$ Hz, H-5′), 6.72 (1H, m, H-6), 6.33 (1H, d, $J = 16.2$ Hz, H-8′), 5.83 (1H, brs, H-5), 4.50 (1H, d, $J = 7.8$ Hz, H-1″), 4.24 (1H, m, H-3), 4.10 (1H, m, H-4), 3.85 (1H, m, H-6″a), 3.65 (1H, m, H-6″b), 3.45 (1H, t, $J = 8.7$ Hz, H-3″), 3.34 (2H, m, H-4″, H-5″), 3.23 (1H, m, H-2″), 2.79 (1H, m, H-2a), 2.30 (1H, m, H-2b). ^{13}C NMR (150 MHz, acetone-d_6 + D_2O): δ 167.3 (C-7), 166.9 (C-9′), 148.2 (C-4′), 146.2 (C-3′), 145.4 (C-7′), 133.0 (C-1), 132.1 (C-2), 126.4 (C-1′), 121.9 (C-6′), 115.5 (C-5′), 114.3 (C-2′), 114.0 (C-8′), 103.6 (C-1″), 78.1 (C-4), 76.8 (C-5″), 76.4 (C-3″), 73.6 (C-2″), 70.2 (C-4″), 67.8 (C-5), 65.9 (C-3), 61.5 (C-6″), 30.7 (C-6).

3.3.2. *2,3-Digalloyl oregonin (**2**)*

Dark yellow amorphous powder. IR (KBr) cm^{-1}: 3346, 1708, 1611, 1526. HR-negative FAB-MS *m/z*: 781.1978 $[M - H]^-$ (calcd $C_{38}H_{37}O_{18}$, 781.1980) ^{1}H NMR (600 MHz, acetone-d_6 + D_2O): δ 7.06 (2H, s, H-2‴′, 6‴′), 7.04 (2H, s, H-2‴, 6‴), 6.69 (1H, d, $J = 7.8$ Hz, H-5″, 6.67 (1H, d, $J = 2.4$ Hz, H-2′), 6.67 (1H, d, $J = 7.8$ Hz, H-5′), 6.59 (1H, d, $J = 2.4$ Hz, H-2″), 6.48 (1H, dd, $J = 2.4$, 7.8 Hz, H-6″), 6.39 (1H, dd, $J = 2.4$, 7.8 Hz, H-6′), 5.32 (1H, m, H-3‴), 5.07 (1H, dd, $J = 7.8$, 9.6 Hz, H-2‴), 4.81 (1H, d, $J = 7.8$ Hz, H-1‴), 4.15 (1H, m, H-5), 4.04 (1H, m, H-5‴a), 3.95 (1H, m, H-4‴), 3.48 (1H, m, H-5‴b), 2.33–2.68 (8H in total, m, H-1, 2, 4, 7), 1.70–1.75 (2H in total, m, H-6). ^{13}C NMR (150 MHz, acetone-d_6 + D_2O): δ 207.7 (C-3), 165.7 (C-7‴′), 165.0 (C-7‴), 145.0 (C-3‴′, 3‴, 5‴′, 5‴), 144.7 (C-3″), 144.6 (C-3′), 143.0 (C-4″), 142.9 (C-4′), 138.1 (C-4‴′), 138.0 (C-4‴), 133.7 (C-1″), 132.8 (C-1′), 120.4 (C-1‴′), 120.3 (C-1‴), 119.4 (C-6′, 6″), 115.3 (C-5′, 5″), 115.1 (C-2″), 115.0 (C-2′), 109.2(C-6‴′, 6‴), 109.1 (C-2‴′, 2‴), 101.2 (C-1‴), 75.7 (C-3‴), 75.2 (C-5), 71.7 (C-2‴), 68.4 (C-4‴), 65.7 (C-5‴), 47.6(C-4), 44.8 (C-2), 37.3 (C-6), 30.5 (C-7), 28.6 (C-1).

3.4. *Measurement of DPPH radical scavenging activity*

Each sample was dissolved in absolute EtOH and added to a DPPH solution (0.1 mM, in absolute EtOH). After mixing gently for 30 min, optical densities were measured at 518 nm using a microplate reader (TECAN, Salzburg, Austria). *L*-ascorbic acid was used as a positive control.

3.5. *Measurement of NBT/superoxide anion scavenging activity*

Each sample was dissolved in 50 mM phosphate buffer (pH 7.5) containing 0.05 mM EDTA, 0.2 mM hypoxanthine, and 0.1 mM NBT. Next, xanthine oxidase (1.2 U/μL) was added to the

mixture. After mixing gently for 30 min, optical densities were measured at 612 nm using a microplate reader (TECAN). Allopurinol was used as a positive control.

3.6. *Statistical analysis*

All data were expressed as the mean ± SD. Values were analysed by Student–Newman–Keuls test, and values of $p < 0.05$ were considered to be significantly different.

4. Conclusion

The activity-guided isolation of *A. sibirica* yielded 10 phenolic compounds (**1**–**10**) including two new compounds, 4-*O*-β-D-glucopyranosyl-5-*O*-caffeoylshikimic acid (**1**) and 2,3-digalloyl oregonin (**2**) which are the first reported shikimic acid conjugated with glucose and caffeic acid (**1**) and diarylheptanoid conjugated with gallotannin (**2**).

The phenolic compounds (**1**–**10**) showed potent antioxidative activities against DPPH and NBT radicals. Especially, **2** and **4** which are diarylheptaoid and **10** which is ellagitannin showed excellent anti-oxidative activities. The results suggest that the leaves of *A. sibirica* and the phenolic compounds isolated from these leaves are promising source of natural products that can be developed as anti-oxidant agents.

Supplementary material

The underlying research materials for this article can be accessed at http://dx.doi.org/10.1080/14786419.2015.1053087

Disclosure statement

No potential conflict of interest was reported by the authors.

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Note

1. These authors contributed equally to this work and should be considered as co-first authors.

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