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Chemical constituents from *Lespedeza cuneata* G. Don (Leguminosae)

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ABSTRACT

Phytochemical investigation of *Lespedeza cuneata* led to the isolation of seventeen compounds including three steroids (β-sitosterol **1**, β-sitosterol-6′-linolenoyl-3-*O*-β-D-glucopyranoside **3**, and β-sitosterol glucoside **13**), nine flavonoids (quercetin **4**, kaempferol **5**, isovitexin **8**, hirsutrin **9**, nicotiflorin **10**, vitexin **11**, astragalin **12**, trifolin **14**, and isorhamnetin **17**), two phenolics (benzyl-β-D-glucopyranoside **7** and homovanillyl alcohol **16**), one carotenoid (loroxanthin **2**), one lignin (7*R*,8*S*—dihydrodehydrodiconiferyl alcohol **15**), and one hexose (pinitol **6**) on the basis of their spectroscopic data. Among these compounds, **2**, **3**, **7**, **15** and **16** were reported for the first time from the genus *Lespedeza*. The taxonomic significance of these isolated compounds was also summarized.

1. Subject and source

Lespedeza is a genus of some 40 species of flowering plants in the leguminosae family, commonly known as bush clover. The genus is native to warm temperature and subtropical regions of eastern North America, eastern and southern Asia, and Australia. *Lespedeza cuneata* is an aggressive, warm-season perennial legume that was introduced from Asia for use in hay production, foraging of poor soils, and controlling erosion along roadsides (Wang et al., 2008). *Lespedeza cuneata* has recently been established as an energy crop to increase the sustainability of agriculture and energy production in the United States (Lau et al., 2004). The aerial parts of this plant have been used to protect liver, kidney, and lung in traditional oriental medicine (Kwon et al., 2007). *Lespedeza cuneata* contains pinitol, tannins, β-sitosterol, and flavonoids including *C*-glycosyl flavones and *O*-glycosyl flavonols (Yoo et al., 2015).

The aerial parts of *L. cuneata* were purchased from Daegu Pharmacopoeia Market, South Korea in 2014. The voucher specimen (No. DS-NPC-003) has been deposited in the Pharmacognosy and Natural Products Chemistry Lab of the College of Pharmacy, Duksung Women's University, South Korea.

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2. Previous work

Previous phytochemical investigations of *L. cuneata* led to the isolation of pinitol, tannins, β-sitosterol, and flavonoids (Matsuura et al., 1978). The flavonoids included *C*-glycosyl flavones such as isoorientin, isovitexin, vicenin II, lucenin II, desmodin, and homoadonivernith and *O*-glycosyl flavonols such as avicularin, juglanin, trifolin, hyperin, and hirsutrin. The aglycones of isolated flavonoids have been reported as quercetin and kaempferol (Yoo et al., 2015).

3. Present study

The aerial parts of *L. cuneata* (3 kg) were extracted with MeOH three times at room temperature to give 300 g of extract. The extract was then dissolved in water and successively partitioned with *n*-hexane, ethyl acetate (EtOAc), and *n*-BuOH to give *n*-hexane-soluble (65.7 g), EtOAc-soluble (54.3 g), and *n*-BuOH-soluble (67.5 g) layers, respectively. The *n*-hexane soluble layer (65.7 g) was subjected to silica gel vacuum liquid chromatography (70–230 mesh) with elution of gradient solvent of *n*-hexane and EtOAc to yield seven fractions (Fr. 1–7). Fr. 3 (4.7 g) was recrystallized under MeOH to yield compound **1** (20 mg). Fr. 5 (11.2 g) was subjected to silica gel column chromatography (CC) eluting with *n*-hexane:EtOAc:MeOH to obtain nine subfractions (5a–5i). Subfractions 5e and 5f were recrystallized under MeOH to yield compound **2** (40 mg). Subfraction 5 h was subjected to silica gel CC (70–230 mesh) eluting with $CHCl_3$:acetone to yield compound **3** (68.5 mg).

The EtOAc-soluble layer (54.3 g) was subjected to silica gel vacuum liquid chromatography with the elution of gradient solvents of CH_2Cl_2 and acetone to yield seven fractions (Fr. A–G). Fr. C (1.2 g) was recrystallized under $CHCl_3$ to give compound **4** (72 mg). Fr. B (1.7 g) was purified by preparative HPLC (Luna 5u C18 100 A column 250 × 10.00 mm, Phenomenex) eluted with CH_3CN–H_2O (flow rate: 2.0 mL/min; 60–65% CH_3CN 15 min, 65–100% CH_3CN 15 min; wavelength 254 nm) to obtain compound **5** (2 mg). Fr. G (10.9 g) was subjected to silica gel CC (70–230 mesh) eluting with CH_2Cl_2:MeOH to give sixteen subfractions (G1–G16). Subfraction G1 was recrystallized under MeOH to yield compound **6** (100 mg). G8 was subjected to preparative HPLC (Luna 5u C18 100 A column 250 × 10.00 mm, Phenomenex) eluted with CH_3CN–H_2O (flow rate: 2.0 mL/min; 15–25% CH_3CN 10 min, 25–50% CH_3CN 40 min, 50–100% CH_3CN 10 min; wavelength 210 nm) to give compound **7** (2.6 mg). G13 was also purified by preparative HPLC (Luna 5u C18 100 A column 250 × 21.20 mm, Phenomenex) eluted with MeOH–H_2O (flow rate: 5.0 mL/min; 40–45% MeOH 10 min, 45% MeOH 7 min, 45–50% MeOH 10 min, 50–100% MeOH 10 min; wavelength 254 nm) to obtain compound **12** (6.8 mg). Fr. F (11.1 g) was subjected to silica gel CC eluting with gradients of CH_2Cl_2 and MeOH to yield eighteen subfractions (F1–F18). F15 was subjected to preparative HPLC (Luna 5u C18 100 A column 250 × 21.20 mm, Phenomenex) eluted with MeOH-water (flow rate: 5.0 mL/min; 40–45% MeOH 10 min, 45% MeOH 7 min, 45–50% MeOH 10 min, 50% MeOH 7 min, 50–55% MeOH 10 min, 55–100% MeOH 10 min; wavelength 254 nm) to obtain compounds **8** (6.5 mg), **9** (24.4 mg), and **10** (2.4 mg). Fr. E (4.6 g) was subjected to reverse phased CC eluted with MeOH and H_2O to yield compounds **11** (16.1 mg), **14** (4.7 mg), and **17** (2.3 mg). Fr. D (3.7 g) was subjected to silica gel CC eluting with $CHCl_3$ and acetone to give thirty-one subfractions (D1–D31). D19 was recrystallized under MeOH to yield compound **13** (10.8 mg). D16 was further purified by preparative HPLC (Luna 5u C18 100 A column 250 × 21.20 mm, Phenomenex) eluted with MeOH–H_2O (flow rate: 5.0 mL/min; 40% MeOH 15 min, 40–45% MeOH 10 min, 45% MeOH 7 min, 45–50% MeOH 10 min, 50% MeOH 7 min, 50–100% MeOH 10 min; wavelength 254 nm) to give compounds **15** (14.5 mg) and **16** (2.4 mg).

The structure of isolated compounds was determined on the basis of MS, ^{1}H NMR, and ^{13}C NMR, compared with data in the literature. Compounds were identified as β-sitosterol (**1**), loroxanthin (**2**) (Maerki-Fischer et al., 1983; Tsutomu et al., 1992), β-sitosterol-6'-linolenoyl-3-*O*-β-D-glucopyranoside (**3**) (Yanjun et al., 2004), quercetin (**4**), kaempferol (**5**), (+)-pinitol (**6**) (Raya-Gonzalez et al., 2008), benzyl-β-D-glucopyranoside (**7**) (Wen et al., 2007), isovitexin (**8**) (Kim et al., 2011), hirsutrin (**9**) (Kohei et al., 2003), nicotiflorin (**10**) (Kohei et al., 2003), vitexin (**11**), astragalin (**12**), β-sitosterol glucoside (**13**), trifolin (**14**) (Kohei et al., 2003), 7*R*,8*S* −dihydrodehydrodiconiferyl alcohol (**15**) (Uddin et al., 2013), homovanillyl alcohol (**16**) (Stella and Photis, 2006), and isorhamnetin (**17**) (Lee et al., 2008) (Fig. 1).

4. Chemotaxonomic significance

In this study, 17 compounds were isolated using chromatographic methods from the extract of aerial parts of *L. cuneata*. To the best of our knowledge, this is the first report of compounds **2**, **3**, **7**, **15**, and **16** from genus *Lespedeza*. Other compounds have previously been isolated from this genus and plant (Yoo et al., 2015).

Various flavonoids and phenolics have been characterized from genus *Lespedeza*. Although flavonoids are predominant in this genus, the type of flavonoids varies depending on species of *Lespedeza*. Prenylated isoflavanones predominate in *Lespedeza bicolor* (Maximov et al., 2004), coumaranochromane derivatives (Ueno et al., 1973) and 3-phenyl benzopyran/benzofuran derivatives predominate in *Lespedeza homoloba* (Miyase et al., 1999), 2-phenylbenzofuran coumestan, flavanones, and flavonols predominate in *Lespedeza vergata* (Chen et al., 2008), dihydrofuranoisoflavanones predominate in *Lespedeza maximowiczi* (Park et al., 2010), and chalcone derivatives, isoflavone, and preticarpan type flavonoids predominate in *Lespedeza cyrtobotrya* (Mori-Hongo et al., 2009). Regarding chemotaxonomy, 5,7,4′-trihydroxy flavones and flavonols could be used as differential markers of *L. cuneata* to distinguish it from others in the genus.

Loroxanthin has been identified as an algae pigment (Garrido et al., 2009). However, the occurrence of loroxanthin in higher plants was identified for the first time in this study. To our knowledge, β-sitosterol-6′-linolenoyl-3-*O*-β-D-glucopyranoside was reported once in grape skin (Yanjun et al., 2004). This is the second report on the occurrence of β-sitosterol-6′-

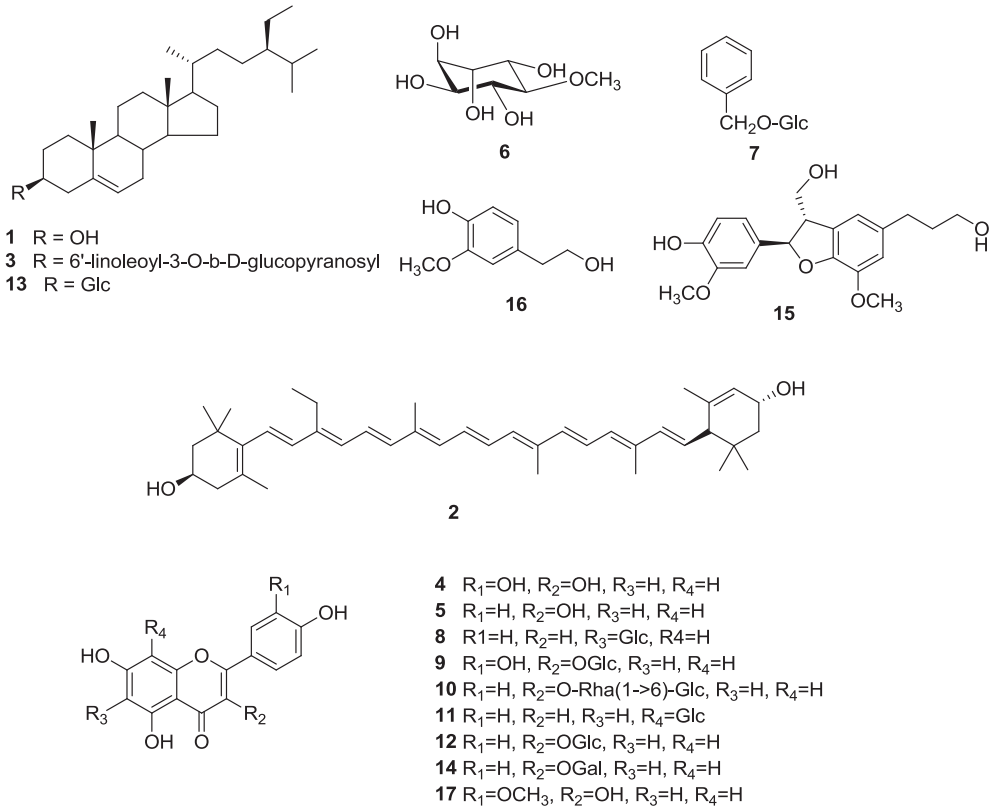

Fig. 1. Chemical structure of compounds **1**–**17**.

linolenoyl-3-*O*-β-D-glucopyranoside in nature, although β-sitosterol and β-sitosterol glucoside have been commonly reported in plants. 7*R*,8*S*–dihydrodehydrodiconiferyl alcohol and benzyl-β-D-glucopyranoside have been identified in many plants (Wu et al., 2012; In et al., 2015), but there have been no reports on their occurrence in this genus or in the family Leguminosae. Homovanillyl alcohol was identified in the family leguminosae in this study for the first time.

In conclusion, the predominant distribution of 5,7,4′-trihydroxy flavones and flavonols may be used to differentiate *L. cuneata* from others in the genus. In addition, the isolated compounds **2**, **3**, **7**, **15** and **16** could be considered chemotaxonomic markers of *L. cuneata* since they have not been previously isolated from any species of *Lespedeza*.

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