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Antioxidant and lipoxygenase inhibitory activity of oligostilbenes from the leaf and stem of *Vitis amurensis*

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ABSTRACT

Ethnopharmacological relevance: The root and stem of *Vitis amurensis* (Vitaceae) have popularly used as traditional medicine for treatment of cancer and various pains in Korea and Japan. Recent studies, its root and stem possess anti-inflammatory, anti-tumor activities, and protective effects against β-amyloid-induced oxidative stress.
Aim of the study: This study deals with the isolation, structural identification of the potent bioactive compounds from the leaf and stem, and their antioxidant capacity, as well as anti-inflammatory effect via lipoxygenase inhibitory assay.
Materials and methods: All isolated compounds yielded after using column chromatography were identified base on the physico-chemical properties and 1D, 2D NMR spectra. The scavenge ability against DPPH and $ABTS^{\bullet+}$ radicals, and to inhibit lipid peroxidation, as well as lipoxygenase type I inhibitory activity of all isolates were performed using *in vitro* assays.
Results: Eleven resveratrol derivatives (**1**–**11**), including a new oligostilbene *cis*-amurensin B (**9**), whose structures were determined on the basis of extensively spectral analyses, were isolated from the leaf and stem of *Vitis amurensis*. The isolates (**1**–**11**) were examined for their antioxidant activities by evaluating scavenge ability against DPPH and $ABTS^{\bullet+}$ radicals, and to inhibit lipid peroxidation. Stilbenes **1** and **4**, and oligostilbenes **5**–**10** displayed moderate anti-lipid peroxidation activities, but all the isolates exhibited strong $ABTS^{\bullet+}$ radical scavenging activity in the dose-dependent manner. In addition, the isolates showed stronger inhibitory capacity against soybean lipoxygenase type I than that of baicalein, a positive control. Of the isolates, *r*-2-viniferin (**8**) exhibited the strongest scavenging activity against $ABTS^{\bullet+}$ radical with TEAC value of 5.57, and the most potential inhibitory effect on soybean lipoxygenase with the IC_{50} value of 6.39 μM.
Conclusion: This is the first report on the potential antioxidant and LOX-1 inhibitory effects of oligostilbenes isolated from the leaf and stem of *Vitis amurensis*. In addition, chemical compositions isolated from the leaf and stem are almost similar to those isolated from the root of *Vitis amurensis*. Therefore, the results may explain, in part, the uses of the leaf and stem, as well as the root of *Vitis amurensis* in the Korean traditional medicine.

1. Introduction

Vitis amurensis (Vitaceae), a wild-growing grape species, is widely distributed in Korea, China, and Japan. The fruits have been used as the raw materials for juice and wine, while the root and stem have been used as traditional medicine for treatment of pains from injury, cancer, stomachache, neuralgic pain, and abdominal pain in Korea and Japan (Huang and Lin, 1999). Recently, it has been reported that the root possesses anti-inflammatory (Huang et al., 2000, 2001), anti-tumor (Lee et al., 2006), anti-aging activities (Lastra and Villegas, 2005), and prevent Alzheimer's disease (Jang et al., 2007). Until now, chemical compositions of the root have been studied on sufficient detail. They include a resveratrol, four dimers and two trimers of resveratrol, amurensin A, (+)-ε-viniferin, ampelopsin A, D, and amurensin B, ampelopsin E, respectively (Huang and Lin, 1999); a resveratrol dimer, amurensin H (Huang et al., 1999a); a resveratrol trimer, amurensin G (Huang et al., 1999b); two resveratrol trimers and two resveratrol pentamers, amurensin C–F, respectively (Huang et al., 2000); 10 resveratrol tetramers, amurensins I–M, (+)-hopeaphenol, vitisin A, (+)-vitisifuran A, and heyneanol A (Huang et al., 2001). However, little is known about phytochemical and bioactive studies of the leaf and stem except

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for a recently report which showed the presence of resveratrol, piceatannol, (+)-ε-viniferin, (−)-ε-viniferin, (+)-ampelopsin A, (+)-isoampelopsin F, and (−)-pallidol in the stem (Kulesh et al., 2006).

Natural antioxidants, majoring phenolic compounds, are considered as important complementary factors for anti-oxidative stress (Saija et al., 1995; Rice-Evans et al., 1997). Interestingly, common stilbenes (*i.e.*, resveratrol, piacetannol, rhapontigenin, etc.) have been found to possess multi-faced bioactivities such as cancer-chemoprevention, anti-inflammation, cardio-protection, and antioxidant properties, inhibition of platelet aggregation (Stojanovic et al., 2001; Roupe et al., 2006). However, the antioxidant and anti-inflammatory activities of oligostilbenes, a special group polymerized from resveratrol or other stilbene units, have been partially known. For this reasons, this paper deals with the isolation, structural identification of a new oligostilbenes (**9**) and 10 known compounds (**1**–**8**, **10**, and **11**) from the leaf and stem of *Vitis amurensis*, and their antioxidant capacity using DPPH, $ABTS^{\bullet+}$, and lipid peroxidation assays. Furthermore, the anti-inflammatory effect via lipoxygenase inhibitory assay of all isolated oligostilbenes was also examined.

2. Materials and methods

2.1. Plant material

The leaf and stem of *Vitis amurensis* were collected at Keryong Mountain in Daejeon, Korea in July 2007. Botanical identification was performed by Prof. K. Bae and the voucher specimen (CNU-1552) was deposited at the Herbarium of the College of Pharmacy, Chungnam National University, Korea.

2.2. Chemicals and reagents

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), DPPH were obtained from Sigma and $K_2S_2O_8$ was obtained from Merck. Lipoxygenase (1.13.11.12) type I-B was purchased from Sigma–Aldrich Chemical Co., USA. All other chemicals were of analytical grade purity.

The organic solvents used in extraction and fractionation were purchased from DaeJung Chemical and Metals Co., Ltd., Korea. All HPLC solvents were ordered from J.T. Baker Analyzed (Mallinckrodt Baker, Inc., Phillipsburg, USA). Water was distilled and filtered through a membrane filters (0.45 μm, USA).

2.3. General experiment

Melting points were determined on an Electrothermal apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were taken in MeOH using a JASCO V-550 UV/VIS spectrometer. IR spectra were recorded on a JASCO 100 IR spectrophotometer. ESI-MS was registered using an HP 1100 series LC/MSD spectrometer. FAB-MS was taken in MeOH and obtained using a JEOL JMS-DX 300 spectrometer. ^1H (100 MHz) and ^{13}C NMR (300 MHz) spectra were recorded on a Bruker DRX300 and Jeol 400 spectrometer (chemical shift values in ^1H and. ^{13}C NMR spectra are presented as δ values with TMS as the internal standard). Two-dimensional (2D) NMR experiments (HMBC, ROESY, COSY) were recorded on a Bruker Advance 600 spectrometer.

Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). UV spots were detected by ultraviolet irradiation (254 and 365 nm) and by spraying 10% H_2SO_4, followed by heating with a heat gun. Column chromatography was performed with silica gel (70–230 and 230–400 mesh, Merck), YMC RP-18 resin (30–50 μm, Fuji Silysia Chemical Ltd.), and Sephadex™ LH-20 (Amersham Biosciences, Uppsala, Sweden). Preparative and analysis HPLC processes were performed on a Shimazu SCL-10A system controller with LC-10AD pump, SPD-10A UV-VIS multi-wavelength detector equipped with a reversed-phase Waters Spherisorb® S5 ODS2 column (USA, 10 mm × 250 mm) and a reversed-phase HICHROM column, C18 (UK, HI-5C18-250A, 4.6 mm × 250 mm, 5 μm), respectively.

2.4. Extraction and isolation

Dried leaf and stem (4.6 kg) of *Vitis amurensis* were extracted with MeOH at room temperature and the combined extract was filtered and concentrated to yield a crude extract (658 g). This extract was suspended in H_2O and then successively partitioned with hexane, EtOAc, and BuOH to afford a hexane-soluble fraction (106 g), an EtOAc-soluble fraction (185 g), and a BuOH-soluble fraction (149 g). The EtOAc fraction exhibited significant antioxidant activity on DPPH, $ABTS^{\bullet+}$, lipid peroxidation, and lipoxygenase inhibitory activity with inhibition percent values of 73.2% (at 62.5 μg/mL), 82.4% (at 10 μg/mL), 66.6% (at 50 μg/mL), and 78.5% (at 50 μg/mL), respectively. Separation of this fraction using a silica gel column (15 cm × 80 cm) and eluted with a gradient of hexane–ethyl acetate to provide seven fractions (Fr. 1–7). Fractions Fr. 4–6, found to be most active, were used for further study. Nine known compounds, piacetannol (**1**) (214 mg), (+)-ampelopsin A (**2**) (563 mg), (+)-ampelopsin F (**3**) (114 mg), resveratrol (**4**) (3000 mg), amurensin G (**5**) (500 mg), gnetin H (**6**) (20 mg), *trans*-ε-viniferin (**7**) (1900 mg), *r*-2-viniferin (**8**) (300 mg), *trans*-amurensin B (**10**) (300 mg), were obtained from Fr. 4–6, using silica gel, LH-20, and reverse phase column chromatographies. A new compound **9** (5.6 mg) was yielded from Fr. 6 using HPLC manner SPD-10A UV-VIS multi-wavelength detector, a reversed-phase Waters Spherisorb® S5 ODS2 column (USA, 10 mm × 250 mm), ACN 40%. The BuOH soluble fraction was divided into five fractions (Fr. A–E) using a Dianion HP-20 CC eluted with H_2O, and MeOH–H_2O (25%, 50%, 75%, 100%) as eluents. Following the TLC guide, Fr. B (12.1 g) was further performed on the gel chromatography column (LH-20, MeOH) to give four subfractions (Fr. B1–B4). Fr. B.2 (5.3 g) was further subjected to a RP column [YMC C-18; MeOH–H_2O (1:3)] to give three sub-fractions (Fr. B.2.1–3). Comp. **11** (140 mg) was received from Fr. B.2.3.3 (1.2 g) by crystallization in MeOH–H_2O (1:4).

Compound **9** was obtained as brown amorphous powder, m.p. 209–211 °C; $[\alpha]_D^{25}$ − 43.2 (MeOH, c 0.5); EIMS *m/z* 680 $[M]^+$; HRES-IMS *m/z* 680.2060 $[M]^+$ (calcd. for $C_{42}H_{32}O_9$); UV [λ_{max} (log ε): 201 (2.91), 208 (3.14), 212 (3.83), 216 (3.76) nm]; IR ν_{max} (3417, 2920, 1609, 1512, 1443, 1410, 1132, 1001 cm^{-1}).

2.5. Analysis by HPLC

Analytical HPLC system was performed on a Shimazu SCL-10A system controller with LC-10AD pump, SPD-10A UV-VIS multi-wavelength detector, a reversed-phase HICHROM column, C18 (UK, HI-5C18-250A, 4.6 mm × 250 mm, 5 μm). The mobile phase consisted of acetonitrile (B), and 0.1% formic acid dissolved in water (A) with a flow rate of 0.9 ml/min. Elution was performed with the following gradient: 0–20 min, hold on 10% B, 22–21 min. linearly increase B from 10% to 20%, 21–50 hold on 20%, 51–75 min hold on 25% B, 756–76 min. linearly increase B from 25% to 30%, 76–120 min hold on 30% B, 121 linearly increase B from 30% to 100%, 122–130 min hold on 100% B. The pH value of buffer was 4.0. The UV spectra were recorded from 190 to 400 nm, and the monitor wavelength was set at 284 nm. Retention time (t_R, min) for compound **1**: 33.8, **2**: 36.7, **3**: 45.1, **4**: 53.8, **5**: 71.0, **6**: 79.3, **7**: 86.7, **8**: 94.2, **9**: 100.2, and **10**: 102.6.