Natural Product Sciences
15(1) : 50-54 (2009)

Quantitative Determination of Diterpenoids from the Roots of *Aralia cordata*

Mi Kyoung Lee, Tran Manh Hung, Byung-Sun Min*, IkSoo Lee[1], MinKyun Na[2], Mi Hee Woo, Jong Keun Son[2], Young Ho Kim[1], Jae Sue Choi[3], and KiHwan Bae[1]

College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea
[1]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
[2]College of Pharmacy, Yeungnam University, Gyeongbuk 712-749, Korea
[3]Faculty of Food Science and Biotechnology, Pukyoung National University, Busan 608-737, Korea

Abstract – A simple and reliable reversed phase HPLC method was developed to determine pharmacologically active compounds *ent*-continentalic acid, kaurenoic acid, and continentalic acid from the roots of *Aralia cordata.* Quantitative analysis of diterpenoids of *A. cordata* were performed by reverse phase C-18 column using a isocratic of acetonitrile : 0.1% trifluoroacetic acid (70 : 30) with UV detection at 205 nm. The correlation coefficients of each calibration curve of *ent*-continentalic acid, kaurenoic acid, and continentalic acid were 0.9987, 0.9983, and 0.9986, respectively. The detection limit of *ent*-continentalic acid, kaurenoic acid, and continentalic acid were 0.5~ 1.0 μg/mL, respectively. The contents of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) of the roots of *A. cordata* collected from seventeen district markets in Korea were 0.09~0.75 mg/g, 1.09~5.43 mg/g, and 2.69~9.08 mg/g, respectively.
Keywords – *Aralia cordata*, HPLC, *ent*-continentalic acid, kaurenoic acid, continentalic acid

Introduction

Aralia cordata Thunb. (Araliaceae) is a medicinal plant, which is distributed in Korea, China, and Japan. The root of *A. cordata* has been used for the treatment of rheumatism, lumbago, and lameness (Perry, 1980). A number of diterpenes and essential oils have been reported from the roots of *A. cordata* (Kim and Kang, 1998; Kim *et al*., 1995), some of which have shown cytotoxic and anti-inflammatory activities (Han *et al*., 1983; Okuyama *et al*., 1991; Seo *et al*., 2007; Dang *et al*., 2005), and antibacterial activities (Kwon and Lee, 2001). Recently, eleven saponins, a number of terpens and polyacetylenes have been isolated and identified from the aerial parts of this plant (Yoshihara and Hirose, 1973; Ito *et al*., 1978; Sawamura *et al*., 1989). Among them, cytotoxic activity of polyacetylenes, as well as anti-bacterial activity of farcarindiol, dehydrofalcarindiol and *ent*-pimara-8(14),15-dien-19-oic acid have been reported (Park and Kim, 1995; Lee *et al*., 2006; Kwon and Lee, 2001). Also, 70% ethanol extract of this plant inhibited the cartilage and chondrocyte destruction through the down-regulation of MMPs activities, and the inhibition of chondrocyte apoptosis through the down-regulation of JNK and p38 MAP kinase signal (Baek *et al*., 2006). This study quantified the contents of *ent*-continentalic acid, kaurenoic acid, and continentalic acid from the roots of *A. cordata* by HPLC method using reverse phase C-18 column.

*Author for correspondence
Tel: +82-53-85036132; E-mail: bsmin@cu.ac.kr

Experimental

General – The chromatographic system for quantitative analysis consisted of a 306 pump (Gilson, USA), 811C dynamic mixer (Gilson, USA), UV/VIS-156 detector (Gilson, USA), 231 XL sample injector (Gilson, USA), and GILSON UniPoint data processor (Gilson, USA). Separation was performed using an Agilent Eclipse XD8-C18 (Agilent Technologies, USA; 5 μm, 4.6 × 150 mm). Methanol (Burdick & Jackson, USA) and acetonitrile (Burdick & Jackson, USA) used in this work were of HPLC grade and other reagents were of analytical grade. Milli-Q (Millipore, MA, USA) treated water (with resistivity more than 17.5 MΩ cm) was used throughout the experiments. Trifluoroacetic acid was purchased from Sigma Chemicals (St. Louis, MO, USA).

Plant material – The roots of *A. cordata* were purchased from oriental medicinal markets, such as, ACC-1 (supplied

from KFDA, cultured at Moojoo), ACC-2 (supplied from KFDA, cultured at Jinan), ACC-3 (supplied from KFDA, cultured at Imsil), ACC-4 (Sehwa-dang, Kwangju, cultured at Imsil), ACC-5 (Johwa Co., Kwangju, cultured at Pyeongchang), ACC-6 (Kyeongdong Co., Geumsan, cultured at Imsil), ACC-7 (Youngdae Co., Geumsan, cultured at Imsil), ACC-8 (Kyungdong-mart, Seoul, cultured at Imsil), ACC-9 (Hyundae Co., Yeongchun, cultured at Imsil), ACC-10 (Gunjae Co., Yeongchun, cultured at Imsil), ACC-11 (Kyungil Co., Yeongchun, cultured at Imsil), ACC-12 (Daebo Co., Daegu, cultured at Andong), ACC-13 (Silla Co., Daegu, cultured at Imsil), ACC-14 (Daeheung-dang, Daegu, cultured at Uiseong), ACC-15 (Kyeongdong Co., Daejeon, cultured at Imsil), ACC-16 (Joongdo Co., Daejeon, cultured at Imsil), and ACC-17 (Baekje-dang., Daejeon, cultured at Imsil).

Isolation of standard compounds – The roots (12 kg) of *A. cordata* were refluxed with MeOH for three hours (3 × 20 L). The total filtrate was concentrated to dryness in vacuum at 40°C in order to render the MeOH extract (3.3 kg) and this extract was suspended in 10% MeOH and sequentially partitioned with hexane (360 g), CH_2Cl_2 (22 g), EtOAC (20 g) and BuOH (237 g), and H_2O (2600 g) in sequence. The hexane-soluble fraction was chromatographed on silica gel column. Gradient elution with hexane-EtOAc and EtOAc-MeOH obtained 15 fractions. Fraction 3 (197 g) was chromatographed on a silica gel column using stepwise gradient eluting with hexane-EtOAc (10 : 1 → 0 : 1) to afford compounds **3** (10145 mg), **2** (736 mg), and **1** (38 mg).

***ent*-Continentalic acid (1)** – light yellow powder; mp, 198 - 202°C; $[\alpha]_D^{25}$ –50° (*c* 0.448, $CHCl_3$); ^{1}H- and ^{13}C-NMR data were consistent with the literature values (Xie *et al*., 1989).

Kaurenoic acid (2) – colorless crystals; mp 178 - 180 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.66, 4.60 (each 1H, s, H-17), 2.50 (1H, brs, H-13), 1.84-2.02 (4H, m, H-3a, H-6a, H-13, H-14a), 1.69-1.77 (4H, m, H-1a, H-2a, H-6b, H-7a), 1.40-1.48 (4H, m, H-2b, H-11a, H-12a, H-12b), 1.12 (2H, brd, *J* = 6.3 Hz, H-15), 1.11 (3H, s, H-18), 1.00 (1H, dd, *J* = 4.6, 7.2 Hz , H-14b), 0.92 (1H, t, *J* = 7.0 Hz, H-5), 0.89 (1H, m, H-3b), 0.85 (1H, m, H-9), 0.82 (3H, s, H-20), 0.68 (1H, m, H-1b); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 185.0 (C-19), 155.8 (C-16), 103.0 (C-17), 57.0 (C-5), 55.1 (C-9), 48.9 (C-15), 44.2 (C-8), 43.8 (C-4), 43.7 (C-13), 41.2 (C-7), 40.6 (C-1), 39.7 (C-10), 39.6 (C-14), 37.7 (C-3), 33.1 (C-12), 28.9 (C-18), 21.8 (C-6), 19.1 (C-2), 18.4 (C-11), 15.6 (C-20).

Continentalic acid (3) - colorless needles; mp 165 - 166°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.71 (1H, dd, *J* = 10.6, 17.2 Hz, H-15), 5.14 (1H, br d, *J* = 1.2 Hz, H-14), 4.94 (1H, dd, *J* = 2.2, 11.2 Hz, H-16b), 4.91 (1H, dd, *J* = 2.1, 17.1, H-16a), 2.34 (1H, ddd, *J* = 2.4, 4.2, 13.8 Hz, H-12b), 2.17 (1H, br d, *J* = 13.5 Hz, H-3b), 1.98 (1H, td, *J* = 5.1, 13.8, H-12a), 1.84-1.92 (2H, m, H-6), 1.78-1.82 (1H, m, H-2b,11b), 1.74 (1H, m, H-1b)1.70 (1H, m, H-9), 1.5-1.6 (1H, m, H-7a), 1.46-1.51 (1H, m, H-11a), 1.22-1.32 (1H, m, H-2a), 1.28 (1H, dd, *J* = 3.0, 14.4, H-5), 1.26 (3H, s, H-18), 1.17-1.24 (1H, m, H-7b), 1.05 (1H, dt, *J* = 3.9, 13.5, H-1a, 3a), 1.00 (3H, s, H-17), 0.65 (3H, s, H-20); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 184.6 (COOH), 147.1 (C-15), 137.9 (C-8), 128.0 (C-14), 112.9 (C-16), 56.1 (C-5), 50.5 (C-9), 44.0 (C-4), 39.2 (C-1), 39.2 (C-10), 38.5 (C-13), 37.9 (C-3), 36.4 (C-12), 35.8 (C-7), 29.3 (C-17), 29.2 (C-18), 24.1 (C-6), 19.6 (C-11), 19.2 (C-2), 13.8 (C-20).

Preparation of test sample – Air-dried roots (100 mg) was finely powdered and sonicated with 1 mL EtOH for 2 hours, and filtered with a 0.22 μm pore size. 20 μL Sample solution was diluted with 10 μL internal standard (3-oxo-23-hydroxyolean-12-en-27-oic acid; 1 mg/100 mL EtOH) and 70 μL EtOH. The diluted extract then filtered through 0.22 μm Millipore filter and a 10 μL sample subjected to HPLC analysis.

HPLC analysis – Method for pimarane- and kaurane-type diterpenoids analysis was modified from those previously described (Xu *et al*., 1998) by using a reverse phase system (Supelco SIL-LC-LC-18, 5 μm, 4.6 × 150 mm i.d.). Elution was with acetonitrile-0.1% trifluoroacetic acid (70 : 30). The flow rate was 1 mL/min, and 10 μL aliquots of samples were injected for analysis and UV detection was carried out at 205 nm.

Calibration – Stock solutions (2 mg/mL) of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) isolated from *A. cordata* were prepared individually in ethanol, and different concentrations (8.0, 16.0, 33.0, 66.0, 132.0 μg/mL) of these were loaded onto an HPLC for the preparation of the calibration functions. The calibration function of *ent*-continentalic acid, kaurenoic acid, and continentalic acid calculated with peak area (*y*), concentration (*x*, μg/mL), and mean values (n = 5) ± standard deviation.

Results and Discussion

The optimal mobile phase composition for the analysis of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) from the 95% EtOH extracts of the roots of *A. cordata* was selected by performing several HPLC runs with various concentrations of acetonitrile in

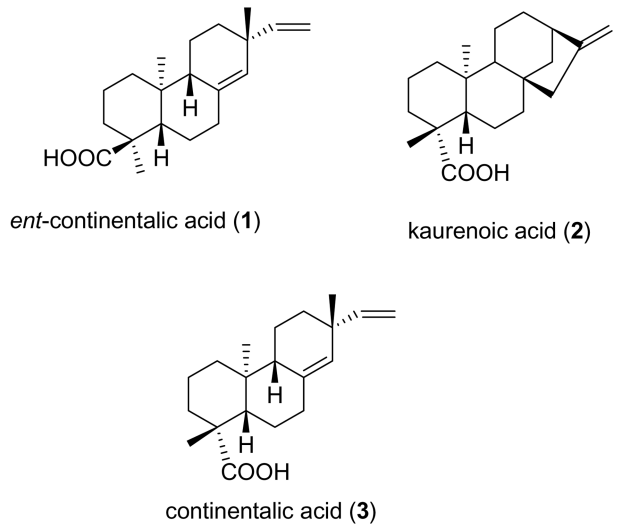

Fig. 1. Structure of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) isolated from *A. cordata*.

acid-buffer solutions (acetic acid, trifluoroacetic acid, phosphoric acid, 10 mM hexansulfonic acid-Na monohydrate) as a mobile phase. Among buffer solutions, 0.1% trifluoroacetic acid could be separated three diterpenoids (**1 - 3**) from *A. cordata* in analytical HPLC system. A solution of 70% acetonitrile in 0.1% trifluoroacetic acid was selected as the mobile phase.

The HPLC peaks of the *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) contained in each roots of *A. cordata* were verified using the standard reference material. The chromatographic system used produced symmetrical peaks with a baseline resolution for *ent*-continentalic acid, kaurenoic acid, and continentalic acid using a simple isocratic profile (Lee *et al*., 2004). 3-Oxo-23-hydroxyolean-12-en-27-oic acid isolated from *Aceriphyllum rossii* (Saxifragaceae) was used as an internal standard. The retention time of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), continentalic acid (**3**), and internal standard were 17.25, 19.45, 21.09, and 24.72 min in the analytical HPLC system, respectively (Fig. 2). Good linearity was achieved in the range from 8.0 to 132.0 μg/mL for three diterpenoids with reverse phase C-18 column. The regression equations of each calibration curve of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) were $y = 0.023614x - 0.04008$, $y = 0.024241x - 0.08218$, and $y = 0.038075x - 0.03807$, respectively, where y is the peak area and x (μg/mL) is the amount of each compound, and correlation coefficients of three compounds (**1 - 3**) were 0.9987, 0.9983 and 0.9986, respectively (Table 1, Fig. 3). Under the above HPLC condition the detection limit of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (3) were 0.5~1 μg/mL (UV_{205} nm), respectively.

The performance of the expressed method was tested by applying it to a simultaneous assay of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) in the roots of *A. cordata* obtained from the seventeen of oriental medicinal markets in Korea. The test samples were prepared as described previously and injected in

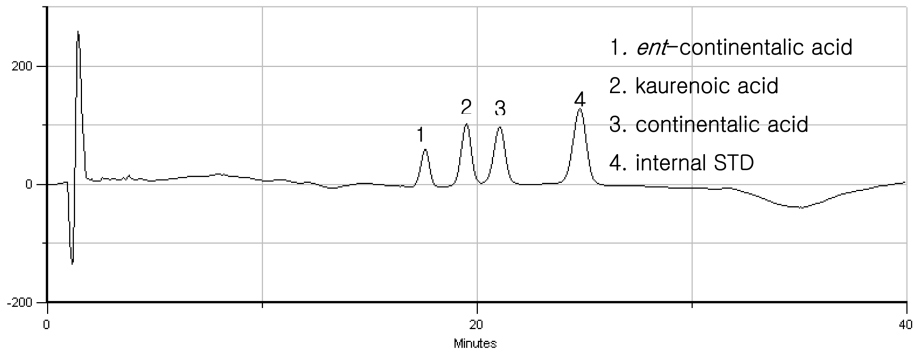

Fig. 2. HPLC chromatogram of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) from *A. cordata*.

Table 1. Calibration graphs, linear ranges, LOD and LOQ of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**)

Compound	linear range (μg/mL)	Slope (a)	Intercept (b)	Correlation Coefficient (r^2)	LOD[a] (μg/mL)	LOQ[b] (μg/mL)
1	8~132	0.023614	−0.04008	0.9987	1	2.5
2	8~132	0.024241	−0.08218	0.9983	1	2.5
3	8~132	0.038075	−0.03807	0.9986	0.5	1.25

[a]Limit of detection. [b]Limit of quality control

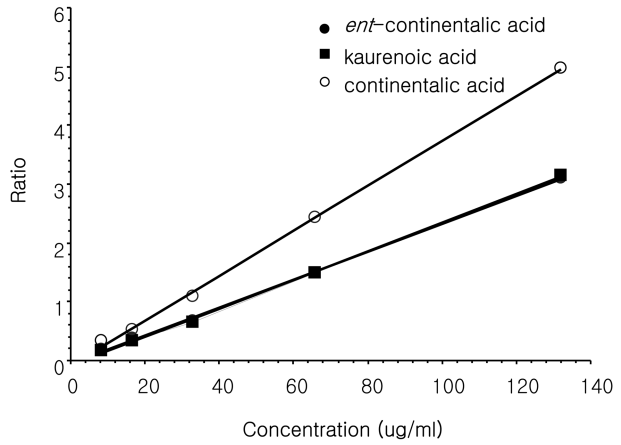

Fig. 3. Calibration curve of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**).

Table 2. Analytical results of *ent*-continentalic acid (**1**), kaurenoic acid (**2**), and continentalic acid (**3**) of *A. cordata* purchased from markets in Korea

Samle	Content (mg/g)		
	ent-continentalic acid (**1**)	kaurenoic acid (**2**)	continentalic acid (**3**)
ACC-1[a]	0.75 ± 0.26	4.55 ± 0.48	7.02 ± 1.00
ACC -2[b]	0.13 ± 0.05	5.43 ± 0.52	8.39 ± 0.61
ACC -3[c]	0.09 ± 0.05	4.31 ± 0.43	6.70 ± 0.24
ACC -4[d]	0.46 ± 0.11	4.09 ± 0.30	7.07 ± 0.72
ACC -5[e]	0.25 ± 0.24	3.77 ± 0.42	6.62 ± 1.66
ACC -6[f]	0.57 ± 0.25	3.08 ± 0.24	4.86 ± 0.08
ACC -7[g]	0.18 ± 0.09	2.03 ± 0.33	2.69 ± 0.61
ACC -8[h]	0.28 ± 0.25	3.93 ± 0.27	5.28 ± 0.53
ACC -9[i]	0.21 ± 0.14	5.60 ± 0.42	5.55 ± 0.53
ACC -10[j]	0.25 ± 0.14	5.26 ± 1.32	6.04 ± 0.37
ACC -11[k]	0.06 ± 0.04	3.54 ± 0.37	5.13 ± 0.23
ACC -12[l]	0.10 ± 0.06	5.22 ± 0.47	7.93 ± 1.04
ACC -13[m]	0.23 ± 0.09	4.46 ± 0.47	6.44 ± 1.00
ACC -14[n]	0.30 ± 0.10	6.10 ± 1.33	9.08 ± 1.66
ACC -15[o]	0.08 ± 0.06	2.44 ± 0.35	3.12 ± 0.27
ACC -16[p]	0.18 ± 0.08	5.14 ± 0.59	6.37 ± 0.59
ACC-17[q]	0.29 ± 0.12	6.06 ± 1.55	8.89 ± 1.35

[a]ACC-1 (supplied from KFDA, cultured at Moojoo), [b]ACC-2 (supplied from KFDA, cultured at Jinan), [c]ACC-3 (supplied from KFDA, cultured at Imsil), [d]ACC-4 (Sehwa-dang, Kwangju, cultured at Imsil), [e]ACC-5 (Johwa Co., Kwangju, cultured at Pyeongchang), [f]ACC-6 (Kyeongdong Co., Geumsan, cultured at Imsil), [g]ACC-7 (Youngdae Co., Geumsan, cultured at Imsil), [h]ACC-8 (Kyungdong-mart, Seoul, cultured at Imsil), [i]ACC-9 (Hyundae Co., Yeongchun, cultured at Imsil), [j]ACC-10 (Gunjae Co., Yeongchun, cultured at Imsil), [k]ACC-11 (Kyungil Co., Yeongchun, cultured at Imsil), [l]ACC-12 (Daebo Co., Daegu, cultured at Andong), [m]ACC-13 (Silla Co., Daegu, cultured at Imsil), [n]ACC-14 (Daeheung-dang, Daegu, cultured at Uiseong), [o]ACC-15 (Kyeongdong Co., Daejeon, cultured at Imsil), [p]ACC-16 (Joongdo Co., Daejeon, cultured at Imsil), [q]ACC-17 (Baekje-dang., Daejeon, cultured at Imsil).

triplicate (Li and Wang, 2004). The results are summarized in Table 2. It was found that the pimarane- and kaurane-type diterpenoids contents of the herbal samples were quite different. Continentalic acid (**3**) is the major compound in the roots of *A. cordata*. Of these roots of *A. cordata*, the sample from Daeheung-dang cultured at Uiseong (ACC-14) had the highest diterpenoid contents (*ent*-continentalic acid, 0.30 ± 0.10 mg/g; kaurenoic acid, 6.10 ± 1.33 mg/g; continentalic acid, 9.08 ± 1.66 mg/g). These results suggest that this method might be used more conveniently for the monitoring the quality of pimarane- and kaurane-type diterpenoids from the roots of *A. cordata*.

Acknowledgement

This work was supported by a grant (08182KFDA254) from the National Center for Standardization of Herbal Medicine funded by the Food Drug and Administration, Republic of Korea (2008).

References

Baek, Y.H., Huh, J.E., Lee, J.D., Choi, D.Y., and Park, D,S., Effect of *Aralia cordata* extracts on cartilage protection and apoptosis inhibition. *Biol. Pharm. Bull.* **29**, 1423-1430 (2006).

Dang, N.H., Zhang, X.F., Zheng, M.S., Son, K.H., Chang, H.W., Kim, H.P., Bae, K.H., and Kang, S.S., Inhibitory constituents against cyclooxygenases from *Aralia cordata* Thunb. *Arch. Pharm. Res.* **28**, 28-33 (2005).

Han, B.H., Han, Y.N., Han, L.A., Park, M.H., and Lee, E.O., Studies on the anti-inflammatory activity of *Aralia continentalis* (I). *Arch. Pharm. Res.* **6**, 17-23 (1983).

Ito, T., Tsukiji, K., and Odagiri, S., Constituents of the head space gas of Udo (*Aralia cordata* Thunb.). *Nippon Nogeikagaku Kaishi* **52**, 223-224 (1978).

Kim, J.S. and Kang, S.S., Saponins from the aerial parts of *Aralia continentalis*. *Nat. Prod. Sci.* **4**, 45-50 (1998).

Kim, Y.H., Kim, H.S., Lee, S.W., Masakazu, U., and Lee J.J., Kaurane derivatives from *Acanthopanax koreanum*. *Phytochemistry* **39**, 449-451 (1995).

Kwon, H.C. and Lee, K.R., Phytochemical constituents of *Artemisia japonica* ssp. *littoricola*. *Arch. Pharm. Res.* **24**, 194-197 (2001).

Lee, I.S., Jin, W.Y., Zhang, X., Hung, T.M., Song, K.S., Seong, Y.H., and Bae, K.H., Cytotoxic and COX-2 inhibitory constituents from the aerial parts of *Aralia cordata*. *Arch. Pharm. Res.* **29**, 548-555 (2006).

Lee, S.M., Park, J.K., and Lee, C.G., Quantitative determination of triterpenoids from the fruits of *Zizyphus jujuba*. *Natural Product Sciences* **10**, 93-95 (2004).

Li, K., and Wang, H., Simultaneous determination of matrine, sophoridine and oxymetrine in *Sophora flavescens* Ait. by high performance liquid chromatography. *Biomed. Chromatogr.* **18**, 178-182 (2004).

Okuyama, E., Nishimura, S., and Yamazaki, M., Analgesic principles from *Aralia cordata* Thunb. *Chem. Pharm. Bull.* **39**, 405-407 (1991).

Park, S.Y. and Kim, J.W., Cytotoxic Polyacetylenes from *Aralia cordata*. *Yakhak Hoeji* **39**, 681-688 (1995).

Perry, L.M., Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; The MIT Press, Cambridge, p. 41 (1980).

Sawamura, M., Kim, M.S., Shichiri, K., Tsuji, T., and Machida, K., Volatile constituents of Japanese and Korean Udo (*Aralia cordata*

Thunb.) and Butterbur (*Petasites japonicus* Miq.). *Research Reports of the Kochi University* **38**, 49-60 (1989).

Seo, C.S., Li, G., Kim, C.H., Lee, C.S., Jahng, Y., Chang H.W., and Son, J.K., Cytotoxic and DNA topoisomearses I and II inhibitory constituents from the roots of *Aralia cordata*. *Arch. Pharm. Res.* **30**, 1404-1411 (2007).

Xie, N., Zhong, S.M., Zjao, S.X., Waterman, P.G., He, C.H., and Zheng, Q.T., Diterpenes from *Pseuduvaria indochinensis*. *J. Chin. Pharm. Univ.* **20**, 203-207 (1989).

Xu, X.D., Zhang, S.M., Zhang, Y.M., Yang, J.S., Wang, C.L., Zhu, Z.Y., and Chen, J.M., Determination of diterpenoic acid, triterpenoids and tritersaponins in *Aralia* by RP-HPLC. *Acta Pharmaceutical Sinica* **33**, 933-936 (1998).

Yoshihara, K. and Hirose, Y., Terpenes from *Aralia* Species. *Phytochemistry* **12**, 468 (1973).

(Accepted March 25, 2009)