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# Ardimerin, a New Dimeric Lactone from the Herb of *Ardisia japonica*

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**Abstract** – A new ardimerin (**1**) was isolated from the herb of *Ardisia japonica*. Its structure was determined by the elucidation of spectroscopic data and a chemical transformation. Compound (**1**) showed potent radical scavenging effect on DPPH radical ($IC_{50}$, 0.32 μM).
**Keywords** – *Ardisia japonica*, ardimerin, DPPH radical scavenging effect

## Introduction

*Ardisia japonica* (Myrsinaceae) is a tropical or subtropical plant, which is widely spread in the seaside area of South Korea, Japan, Taiwan, and China. The fruit and stem of this species have been used as traditional Korean and Chinese medicines to stop cough and uterine bleeding (Perry *et al.*, 1980). Many constituents from *Ardisia* spp. have been reported to have a variety of biological activities such as anti-aging (Takei, 1999), anti-inflammatory (Takei, 1999), skin whitening effect (Kim *et al.*, 2000; Takei, 1999), allelochemical activity (Neal *et al.*, 1998), anti-HIV activity (Piacente *et al.*, 1996), plant growth inhibitory (Lee *et al.*, 1997), 5-lipoxygenase inhibitory (Fukuyama *et al.*, 1993), anti-tubercular (Huang *et al*, 1981 and 1980), and chitin biodegradative effect (Hirano *et al.*, 1996). In recent years, the role of free radical and reactive oxygen species in human diseases has become more apparent. Thus, the studies on antioxidant activity of compounds derived from natural resources have attracted considerable research interest (Gordon, 1996). In the course of our continuing search for anti-aging agents from medicinal plants, we found that the methanol extract of *Ardisia* spp. exhibited potent antioxidant activity assessed by the scavenging effect of DPPH radical *in vitro*. Bioassay-guided fractionation afforded a unique ardimerin (**1**) with radical scavenging effect on DPPH radical. Herein, we describe the isolation, structural elucidation and radical scavenging activity of **1**.

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## Materials and Methods

General-UV spectrum was recorded with a HP8453 UV/VIS spectrophotometer. IR spectrum was performed on a Perkin-Elmer model 1750 FT-IR spectrophotometer. FABMS spectra were measured on a JEOL JMX-SX 102 mass spectrometer. High resolution mass measurement was done with a JEOL AX-505H mass spectrometer using m-NBA as a matrix. ESIMS spectrum was also obtained by Mariner (ABI) mass spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded in $CD_3OD$ or $CDCl_3$ at 25°C on a Brucker ARX-400 NMR spectrometer. Chemical shifts (δ) are given relative to TMS, using the solvent peaks [$CD_3OD$ ($\delta_H$ 3.30, $\delta_C$ 49.0) and $CDCl_3$ ($\delta_H$ 7.26, $\delta_C$ 77.1)] as the internal standards. Analytical TLC and preparative TLC were performed on precoated Kieselgel 60 $F_{254}$ plates (0.25 mm, Merck) and Kieselgel 60 $F_{254}$ plates (0.5 mm, Merck), respectively. The silica gel used for column chromatography was Kieselgel 60 (70-230 mesh, Merck).

**Plant materials** – The whole herb of *Ardisia japonica* was collected in Sunheul, Jeju, Korea. The sample was identified by Professor M. H. Kim of Dep. of Biology, Cheju National University. A voucher specimen (SH203) was deposited at the Laboratory of Natural Products Chemistry, Cheju National University.

**DPPH radical scavenging assay** – Radical scavenging effects on DPPH (α,α'-diphenylpicrylhydrazyl) radical were determined by the method previously described (Ryu *et al.*, 2001).

**Extraction and isolation** – The dried whole herb of

*Ardisia japonica* (1.0 kg) was extracted with MeOH at room temperature for a day. The MeOH extract (28 g) having DPPH radical scavenging effect ($IC_{50}$, 26 μg/mL) was partitioned between 60% MeOH and $CH_2Cl_2$. The former layer was further partitioned between *n*-BuOH (4.8 g, 17.1%) and $H_2O$ (18 g, 64.3%). The bioactive *n*-BuOH fraction was subjected to an ODS flash chromatography with aqueous MeOH (0, 20, 40, 60, 100%). By the estimation of the DPPH radical scavenging activity of each fraction, the most active 40% MeOH fraction (180 mg) was further fractioned by gel-filtration with MeOH to afford a crude phenolic glycoside fraction ($IC_{50}$, 1.6 μg/mL). This fraction was finally purified by reversed-phase HPLC with 54% MeOH to yield ardimerin (1, 24 mg, 0.086%). While the latter layer ($CH_2Cl_2$) was fractionated on ODS flash and silica column chromatography to afford an active fraction. Preparative TLC of this fraction gave four compounds with moderate radical scavenging activity on DPPH radical. Their structural elucidation is in progress.

**Compound 1** – Pale brown solid; $[\alpha]_D^{23}$ +48.2° (*c* 0.62, MeOH); UV $\lambda_{max}$ (log ε) 275 nm (0.22), 216 (1.32) and 197 (3.00); IR (film) $\nu_{max}$ $cm^{-1}$ 3350 (br, OH), 1710 (C=O), 1605 (aromatic), 1210 (C-O), 960; FABMS (pos) *m/z* 657 $[M + H]^+$, 679 $[M + Na]^+$; HRFABMS (pos) *m/z* $[M + H]^+$ 657.4563 (calcd 657.4558 for $C_{28}H_{32}O_{18}$); $^1H$ NMR ($CD_3OD$, 400 MHz) and $^{13}C$ NMR ($CD_3OD$, 100 MHz) see Table 1.

**Acetylation of 1** – A mixture of ardimerin (**1**, 9.0 mg), $Ac_2O$ (1.0 mL) and pyridine (1.0 mL) was stirred at room temperature overnight. The reagents were evaporated *in vacuo* and the residue was subjected to $SiO_2$ column chromatography [$CH_2Cl_2$/MeOH (95:5)] to yield a decaacetylardimerin (**2**, 14 mg).

**Compound 2** – colorless solid; ESIMS (pos) *m/z* 1077 $[M + H]^+$, 1095 $[M + H_2O + H]^+$; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.72 (1H, s), 5.46 (1H, t, *J* = 9.3 Hz), 5.11 (1H, t, *J* = 9.3 Hz), 4.79 (1H, d, *J* = 10.4 Hz), 4.34 (2H, m), 4.15 (1H, br d, *J* = 10.4 Hz), 3.90 (3H, s, $OCH_3$), 3.80 (1H, br d, *J* = 9.5 Hz), 2.33 (12H, s), 2.12 (12H, s), 2.08 (12H, s), 2.05 (6H, s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 170.7, 170.3, 169.9, 168.5, 167.9, 161.8, 150.3, 144.5, 141.6, 129.8, 124.3, 118.9, 73.2, 72.4, 68.8, 62.1, 61.8, 21.0, 20.8.

## Results and Discussion

Compound **1** was obtained as a pale brown solid. The molecular formula of **1** was established as $C_{28}H_{32}O_{18}$ on the basis of HRFABMS and $^{13}C$ NMR data. The IR spectrum of **1** revealed absorptions due to a hydroxyl group (3350 $cm^{-1}$), a carbonyl (1710), an aromatic C=C band (1605), and a C-O band (1210). The $^1H$ and $^{13}C$ NMR spectra of **1** (Table 1) were reminiscent of bergenin (**4**) combined a gallic acid (**3**) with a *C*-glucose (Ramaiah *et al.*, 1979; Taneyama *et al.*, 1983). A variety of 2D NMR experiments clarified the entire carbon framework of **1** to be listed in Table 1. In particular, HMBC cross peaks between H6 and C1', and between H-1' and H-2' and C6 clarified a β-D-glucosyl residue [δ 81.9, 79.9, 73.8, 72.3, 70.8, 61.2; $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 9.8 Hz and $J_{C1'\text{-}H\text{-}1'}$ = 145.2 Hz] (Brakta *et al.*, 1993) was directly linked to *meta* position of phenylcarboxylic acid as a *C*-glycoside linkage. No HMBC correlation between the carboxylic carbon and the H-2' of the glucosyl moiety to lead to δ-lactone as showed in bergenin (**4**) was observed in the HMBC spectrum of **1**. A cross peak between 3-OH and C-4 in the HMBC spectrum measured in DMSO-$d_6$ suggested that a hydroxyl group should be positioned at

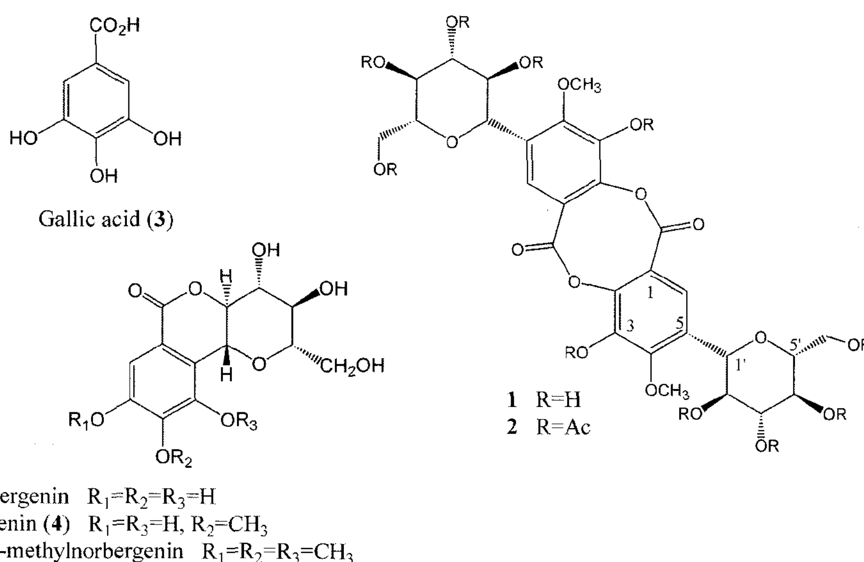

**Table 1.** $^1H$ and $^{13}C$ NMR data of ardimerin (**1**) in $CD_3OD$ at 25°C

| Position | $\delta_C$ | $\delta_H$ ($J$ Hz) | HMBC |
|---|---|---|---|
| C=O | 163.5 | | |
| 1 | 118.2 | | |
| 2 | 151.1 | | |
| 3 | 148.2 | | |
| 4 | 140.7 | | |
| 5 | 116.0 | | |
| 6 | 109.6 | 6.98 (1H, s) | C=O, C2, C4, C5, C1' |
| 4-$OCH_3$ | 59.9 | 3.75 (3H, s) | C4 |
| 1' | 72.3 | 4.94 (1H, d, 9.8) | C5 |
| 2' | 79.9 | 3.98 (1H, t, 9.8) | C5, C3', C1' |
| 3' | 73.8 | 3.65 (1H, m) | C4', C3' |
| 4' | 70.8 | 3.20 (1H, t, 8.9) | C6', C5', C3' |
| 5' | 81.9 | 3.56 (1H, m) | C4', C3' |
| 6' | 61.2 | 3.83 (1H, br d, 11.7) | C5' |
| | | 3.45 (1H, dd, 11.7, 2.4) | |

C-3. Furthermore, the molecular weight [$(M+H)^+$ at $m/z$ 1077] of decaacetylardimerin (**2**) is 748 daltons higher than that of **4**, indicating that **1** is a $C_2$-symmetric dimer. The carbonyl carbon of one side is connected to 2-OH of the opposite each other, finally forming an eight membered bislactone.

Compound **1** showed potent radical scavenging effect on DPPH radical ($IC_{50}$, 0.32 μM). Chen's group and Bouchet's group asserted that the radical scavenging activity is dependent on the position of hydroxyl groups (Chen and Ho, 1997; Bouchet *et al.*, 1998). The presence of a second hydroxyl group in the *ortho* or *para* position increases the antioxidant activity due to additional resonance stability and *o*-quinone or *p*-quinone formation. Two hydroxyl groups of **1** orientated to *ortho* each other, while those of bergenin (**4**) to *meta*.

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## References

Bouchet, N., Barrier, L. and Fauconneau, B., Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae), *Phytother. Res.*, **12**, 159-162 (1998).

Brakta, M., Farr, R. N., Chaguir, B., Massiot, G., Lavaud, C., Andeson, W., Sinou, D. and Daves, G. Jr., Assignment of anomeric configuration of *C*-glucopyranosides and *C*-glucofuranosides. $^1H$, $^{13}C$ and nuclear overhauser enhancement spectrometric study, *J. Org. Chem.*, **58**, 2992-2998 (1993).

Chen, J. H. and Ho, C.-T., Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds, *J. Agric. Food Chem.*, **45**, 2374-2378 (1997).

Fukuyama, Y., Kiriyama, Y., Okino, J., Kodama, M., Iwaki, H., Hosozawa, S. and Matsui, K., Naturally occurring 5-lipoxygenase inhibitor. II. Structures and synthesis of ardisianones A and B, and maesanin, alkenyl-1,4-benzoquinones from the rhizome of *Ardisia japonica*, *Chem. Pharm. Bull.*, **41**, 561-565 (1993).

Gordon, M. H., Dietary antioxidants in disease prevention, *Nat. Prod. Rep.*, **13**, 265-273 (1996).

Hirano, S., Kitaura, S., Shuntaro, S., Nozomi, S., Sugiyama, M., Hashimoto, K. and Tanatani, A., Chitin biodegradation and wound healing in tree bark tissues, *J. Envir. Polym. Degrad.*, **4**, 261-265 (1996).

Huang, B.H., Chen, W. S., Hu, Y., Qiu, C. B., Zhang, W. J. and Liang, B. L., Study on the chemical constituents of *Ardisia japonica* (Hornsted) Blume an antitubercular Chinese herb, *Yao Hsueh Hsueh T'ung Pao*, **16**, 27-30 (1981).

Huang, P. H., Chen, W. S. and Hu, Y., Studies on antitubercular constituents from *Ardisia japonica*, *Yao Hsueh Tung Pao*, **15**, 39-39 (1980).

Kim, C. T., Kim, H. J., Jung, M. H. and Jin, M. H., Skin whitener compositions containing selina-4(14),7(11)-dien-8-one, LG Chemical Ltd., S. Korea, Patent No. 2000071092 (2000).

Lee, A. K. and Suh, J. K., Effect of media, plant growth regulators, and hot water treatment on rooting of stem and root cuttings in *Ardisia* spp., *Han'guk Wonye Hakhoechi*, **38**, 546-550 (1997).

Neal, J. W., Davis, J. C., Bentz, J. A., Warthen, J. D., Griesbach, R. J. and Santamour, F. S., Allelochemical activity in *Ardisia* species (Myrsinaceae) against selected arthropods, *J. Econ. Entom.*, **91**, 608-617 (1998).

Perry, L. M. and Metzger, J., Medicinal plants of East and South Asia, The MIT Press, Cambridge, MA (1980).

Piacente, S., Pizza, C., Tommasi, N. and Mahmood, N., Constituents of *Ardisia japonica* and their *in vitro* anti-HIV activity, *J. Nat. Prod.*, **59**, 565-569 (1996).

Ramaiah, P. A., Row, L. R., Reddy, D. S. and Anjaneyulu, S. R., Isolation and characterization of bergenin derivatives from *Macaranga peltata*, *J. Chem. Soc., Perkin Trans. I*, 2313-2316 (1979).

Ryu, G., Park, E. K., Joo, J. H., Lee, B. H., Choi, B. W., Jung, D. S. and Lee, N. H., A new antioxidant monoterpene glycoside, α-benzoyloxypaeoniflorin from *Paeonia suffruticosa*, *Arch. Pharm. Res.*, **24**, 105-108 (2001).

Takei, M., Skin external use agent, Noevir Co., Ltd., Japan, Patent No. JP11171784 (1999).

Taneyama, M., Yoshda, S., Kobayashi, M. and Hasegawa, M., Isolation of norbergenin from *Saxifraga stolonifera*, *Phytochem.*, **22**, 1053-1054 (1983).

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