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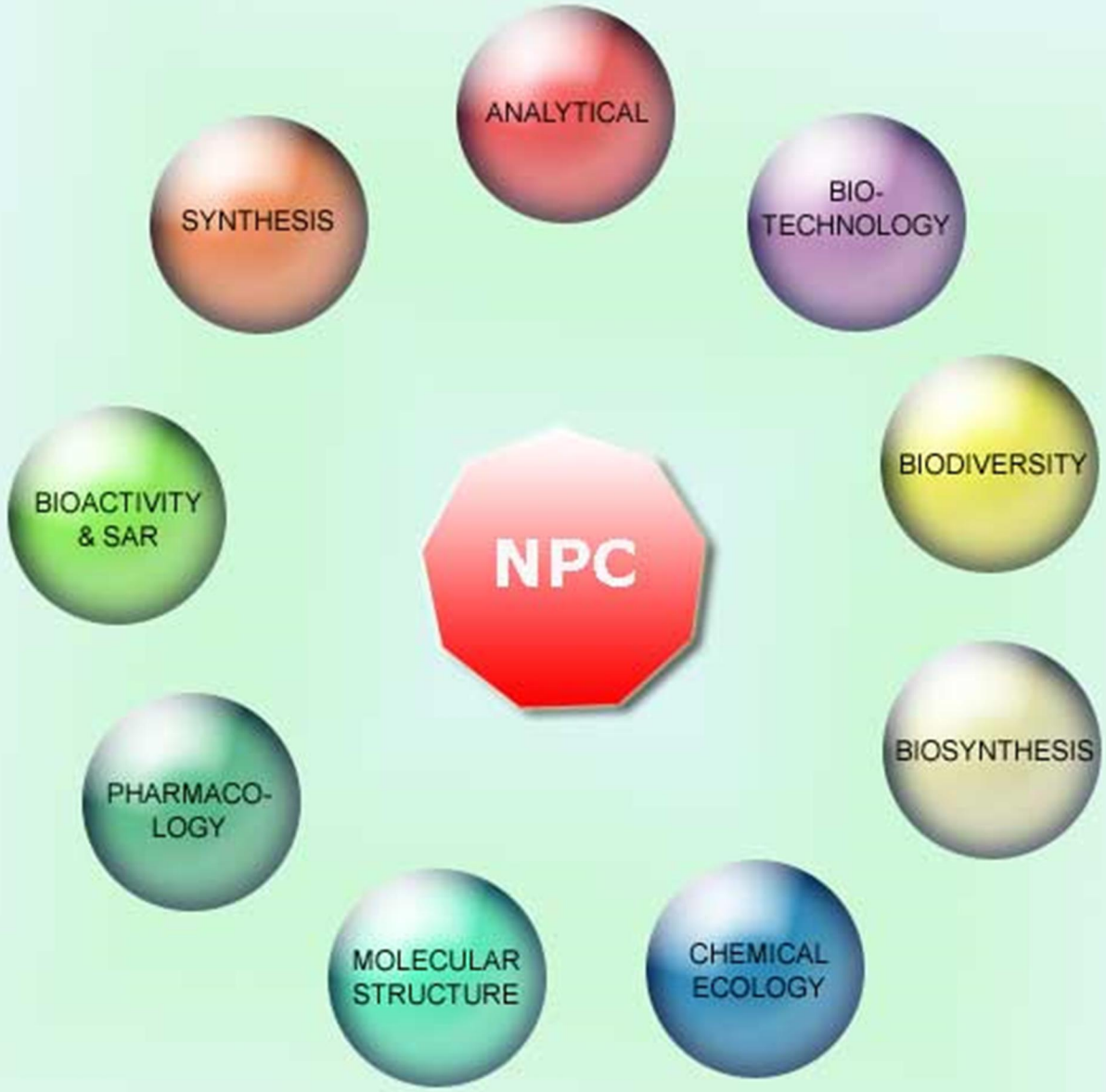
An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 10. Issue 11. Pages 1797-2018. 2015
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

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NPC Natural Product Communications

2015
Vol. 10
No. 11
1929 - 1932

Bioassay-guided Isolation of Antiproliferative Triterpenoids from *Euonymus alatus* Twigs

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Received: March 18th, 2015; Accepted: July 16th, 2015

Euonymus alatus (Celastraceae) has been used as an anticancer agent in Korean traditional medicine. However, the potential bioactive contributors to the anticancer effects have not been thoroughly studied. Our screening test revealed that the MeOH extract of *E. alatus* twigs exhibited significant cytotoxicity against A549, SK-OV-3, and SK-MEL-2 cell lines. A bioassay-guided separation of the MeOH extract of *E. alatus* twigs resulted in the isolation and identification of 14 triterpenes as main phytochemicals. The structures of the compounds were elucidated on the basis of spectroscopic evidence as lupeol (**1**), betulin (**2**), 3*β*,28,30-lup-20(29)-ene triol (**3**), lupenone (**4**), betulone (**5**), 28,30-dihydroxy-3-oxolup-20(29)-ene (**6**), messagenin (**7**), glut-5-en-3*β*-ol (**8**), maslinic acid (**9**), hederagenin (**10**), 3-oxo-11*α*-methoxyolean-12-ene (**11**), 3*β*-hydroxy-1-oxo-olean-12-en-28-oic acid (**12**), ursolic acid (**13**), and 2*α*-hydroxy-ursolic acid (**14**). Of these compounds, **3**, **6-8**, and **10-14** were isolated for the first time from this plant. All isolated triterpenoids had consistent antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines. Compounds **2**, **5**, and **7** showed significant cytotoxicity against all four cell lines tested, with IC_{50} values of 3.26-8.61 μM.

Keywords: *Euonymus alatus*, Celastraceae, Triterpenoid, Antiproliferation, Structure-activity relationships.

Triterpenoids represent a large and diverse group of organic compounds characterized by the basic backbone of a 30-carbon isoprenoid molecule, and are ubiquitously distributed throughout the plant kingdom [1]. Because of the growing importance of triterpenoids as a source of therapeutic medications for various chronic diseases, there have been many studies investigating the potential biological activities and possible molecular targets of natural triterpenoids as well as synthetic triterpenoids [2-5]. Recent experimental evidence supports the beneficial effects of triterpenoids as potential agents for the prevention and treatment of various types of cancer [5,6]. There is also convincing evidence in experimental animal models demonstrating the anti-carcinogenic effects of triterpenoids against several cancer types, mainly by modulating the pathways that contribute to cell proliferation and apoptosis [5,6].

Euonymus alatus (Thunb.) Sieb. (Celastraceae) is a deciduous and popular ornamental tree that is commonly known as winged euonymus. The cork cambium on the twigs of this tree, which is called 'gui-jun woo', has long been used to treat cancers in Korean traditional medicine [7]. Recently, pharmacological studies have supported the potential of *E. alatus* as an anticancer agent using a variety of bioactive evaluations *in vivo* and *in vitro* models [8-10]. Known bioactive constituents of *E. alatus*, including sesquiterpenes, sesquiterpene alkaloids, triterpenes, flavonoids, and phenolic compounds have been reported [11-15]. However, to the best of our knowledge, the bioactive compounds that contribute to its anticancer effects have not been completely studied.

The MeOH extract of *E. alatus* twigs showed significant cytotoxicity against A549, SK-OV-3, and SK-MEL-2 cell lines based on the results of a sulforhodamine B (SRB) bioassay [16,17].

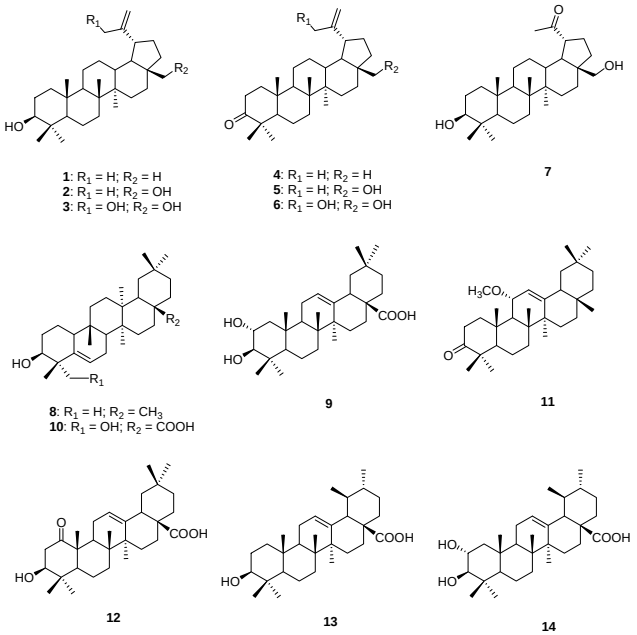

Figure 1: Chemical structures of the isolated triterpenoids **1-14**.

As part of a search for the antiproliferative constituents from the twigs of *E. alatus*, we previously reported on the isolation and identification of five new phenolic compounds with cytotoxicity as well as anti-neuroinflammatory activity [16]. In the course of our continuing search for anticancer compounds from *E. alatus* twigs, we further investigated the active constituents of its MeOH extract based on a bioactivity-guided isolation principle. In the present

research, we report the isolation and structural elucidation of 14 triterpenes (**1-14**) from the active $CHCl_3$-soluble fraction as the main phytochemicals (Figure 1). The SRB bioassay was performed to evaluate the antiproliferative activities of compounds **1-14** by determining their inhibitory effects on human tumor cell lines A549, SK-OV-3, SK-MEL-2, and HCT-15. In addition, the structure-activity relationships (SAR) of the isolated triterpenoids on the antiproliferative effects were addressed in this study.

Based on the bioactivity-guided isolation principle, the methanolic crude extract of *E. alatus* twigs was fractionated to yield soluble fractions of *n*-hexane, $CHCl_3$, *n*-BuOH, and water. As shown in Table 1, the crude MeOH extract displayed significant cytotoxicity against A549, SK-OV-3, and SK-MEL-2 cells, with IC_{50} values of 8.2, 6.6, and 8.4 μg/mL, respectively. Among the derived soluble fractions, the *n*-hexane and $CHCl_3$ soluble fractions had cytotoxicity against A549, SK-OV-3, and SK-MEL-2 cells, while the *n*-BuOH and water soluble fractions were inactive (IC_{50}: >50.0 μg/mL) (Table 1). The cytotoxic effect of the $CHCl_3$ soluble fraction (IC_{50} value of 8.1-8.6 μg/mL) was higher than that of the *n*-hexane soluble faction (IC_{50} value of 24.6-27.0 μg/mL), which led us to investigate the $CHCl_3$ soluble fraction for active compounds. Further fractionation of the $CHCl_3$ soluble fraction using column chromatography revealed that its derived fractions A1, A2, A3, and A4 showed considerable cytotoxic activity against the tested cell lines (Table 1). The active fractions A1, A2, and A3 were consolidated, and the combined fraction was applied to RP-C_{18} silica gel column chromatography, which further afforded seven active fractions; B1, B9, B10, and B12-B15 (Table 1). Fraction A4 was subjected to a Sephadex LH-20 column, which provided additionally three active fractions; C3, C4, and C5. Semi-preparative HPLC purification of the active fraction B1 yielded a lupane-type triterpene **4** and an oleanane-type triterpene **11**. Similarly, the active fraction B9 afforded two lupane-type triterpenes **3** and **7**, and a lupane-type triterpene **6** was isolated from the active fraction B10; an oleanane-type triterpene **8** from the active fraction B12; a lupane-type triterpene **5** from the active fraction B13; a lupane-type triterpene **2** from the active fraction B14; a lupane-type triterpene **1** and an ursane-type triterpene **13** from the active fraction B15. Finally, three oleanane-type triterpenes **9**, **10**, and **12** and an ursane-type triterpene **14** were obtained from the consolidated fractions C3, C4, and C5. The structures of the isolated triterpenoids **1-14** were unambiguously confirmed by spectroscopic methods, including ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS analysis. The compounds were identified as lupeol (**1**) [18], betulin (**2**) [19], 3*β*,28,30-lup-20(29)-ene triol (**3**) [20], lupenone (**4**) [21], betulone (**5**) [22], 28,30-dihydroxy-3-oxolup-20(29)-ene (**6**) [23], messagenin (**7**) [24], glut-5-en-3*β*-ol (**8**) [25], maslinic acid (**9**) [26], hederagenin (**10**) [27], 3-oxo-11*α*-methoxyolean-12-ene (**11**) [28], 3*β*-hydroxy-1-oxo-olean-12-en-28-oic acid (**12**) [29], ursolic acid (**13**) [30], and 2*α*-hydroxyursolic acid (**14**) [26] by comparing their obtained spectroscopic data with previously reported values. To the best of our knowledge, compounds **3**, **6-8**, and **10-14** were isolated for the first time from this plant.

The sulforhodamine B cell proliferation assay was used to evaluate the antiproliferative activities of the isolated triterpenes **1-14** [17], which were tested against four human tumor cell lines, including A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). The IC_{50} (the concentration that causing 50% inhibition of cell proliferation) values of each compound were determined and are summarized in Table 2. The data demonstrated that all isolated triterpenoids displayed consistent antiproliferative activities against all of the cell lines with IC_{50} values ranging from 3.3 to 27.5 μM, except that no cytotoxic effects were observed for compounds **8** and **11** against SK-OV-3, SK-MEL-2, and HCT-15 cells (IC_{50} >30.0 μM). Especially, compounds **2**, **5**, and **7** showed the most potent cytotoxicity against all four cell lines with IC_{50} values ranging from 3.3-8.6 μM.

Table 1: Cytotoxicity of the methanolic extract and its derived soluble fractions from *E. alatus* twigs against three human cancer cell lines.

Fractions	IC_{50}, μg/mL[a]		
	A549	SK-OV-3	SK-MEL-2
Crude MeOH extract	8.2	6.6	8.4
n-Hexane soluble fraction	27.0	24.6	26.9
$CHCl_3$ soluble fraction	8.4	8.6	8.1
n-BuOH soluble fraction	>50.0	>50.0	>50.0
Water fraction	>50.0	>50.0	>50.0
Fraction A1	9.8	11.8	9.9
Fraction A2	10.3	12.3	9.1
Fraction A3	7.1	6.7	5.9
Fraction A4	15.7	12.8	13.0
Fraction A5	>50.0	40.8	>50.0
Fraction A6	>50.0	37.2	>50.0
Fraction A7	>50.0	>50.0	>50.0
Fraction B1	25.40	17.8	12.3
Fraction B9	8.4	14.1	12.5
Fraction B10	8.2	8.8	11.9
Fraction B12	27.0	25.4	20.2
Fraction B13	5.2	6.8	5.3
Fraction B14	4.9	5.2	6.4
Fraction B15	8.7	9.3	6.3
Fraction C3	21.1	17.4	18.7
Fraction C4	15.7	12.7	18.2
Fraction C5	16.7	8.3	23.7
Etoposide[b]	1.9	1.8	1.2

[a]IC_{50} value of extract and fractions against each tested cell line, which was defined as the concentration (μg/mL) that caused 50% inhibition of cell growth *in vitro*. [b]Etoposide served as a positive control.

Table 2: Antiproliferative effects of compounds **1-14** against four cultured human tumor cell lines using the SRB bioassay *in vitro*.

Compounds	IC_{50}, μM[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	17.1	15.7	10.9	13.7
2	3.9	7.5	4.1	8.6
3	14.5	13.3	14.5	20.7
4	15.7	12.2	9.2	10.4
5	3.6	5.1	3.3	3.4
6	11.9	13.7	10.8	6.5
7	4.1	8.6	3.4	7.2
8	27.5	>30.0	>30.0	>30.0
9	16.5	18.7	14.7	21.9
10	18.8	21.1	13.8	18.6
11	25.5	>30.0	>30.0	>30.0
12	13.5	15.1	11.2	13.9
13	11.8	13.8	11.9	17.7
14	13.3	15.3	12.1	12.8
Doxorubicin[b]	0.002	0.012	0.002	0.129

[a]IC_{50} value of compounds against each tumor cell line. Each value is the mean of three values. [b]Doxorubicin served as a positive control.

Three types of triterpenoids, including lupane-type (**1-7**), oleanane-type (**8-12**), and ursane-type (**13-14**) triterpenoids were tested for their antiproliferative activities. It is interesting to note that the order of average cytotoxicity of the isolated triterpenoids from high to low can be listed as lupane-type > ursane-type > oleanane-type. Even though all the active triterpenes showed broad spectrum activity, compounds **2**, **5**, and **7** with a hydroxyl group at C-28 demonstrated the most significant cytotoxicity against all four cell lines tested. Other lupane-type triterpenes showed moderate antiproliferative activities. These results suggest that the presence of the hydroxyl group at C-28 on lupane-type triterpenoids seems to be necessary to exert a good antiproliferative activity. However, the presence of the hydroxyl group at C-30 on lupane-type triterpenoids might not always increase cytotoxic activity. New information about structure-activity relationship was obtained by comparing cytotoxicity of **1** with **4**, **2** with **5**, and **3** with **6**, in which paired compounds have the same skeleton. This led to the information that the existence of a ketone group at C-3 on lupane-type triterpenoids induced cytotoxicity slightly more effectively than the existence of

a hydroxyl group at C-3. This may be associated with lipophilicity, and a higher lipophilicity may facilitate penetration through the lipophilic plasma membrane of the tumor cells, and subsequently, to a higher cytotoxicity [31]. On the other hand, oleanane-type triterpenes **8** and **11** showed selective cytotoxic effects against A549 weakly, while other compounds **9**, **10**, and **12** showed high cytotoxic activity against all of the tested cell lines. This led us to conclude that the presence of a carboxyl group at C-28 is essential for the cytotoxic activity of the oleanane-type triterpenes. The ursane-type triterpenes **13** and **14** exhibited moderate antiproliferative activities against four human tumor cell lines.

In the present study, the MeOH extract of *E. alatus* twigs was investigated to explore its antiproliferative constituents. All of the isolated triterpenes showed significant antiproliferative activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines, which confirmed that the isolated triterpenoids represented a portion of the molecules associated with anticancer effects of *E. alatus*. In conclusion, our results strongly suggest that the MeOH extract of *E. alatus* twigs is a potential source of anticancer health products, which supports the ethnopharmacological use of *E. alatus* in Korean traditional medicine to treat various cancers.

Experimental

General experimental procedures: Optical rotations were measured on a Jasco P-1020 Polarimeter, IR spectra on a Bruker IFS-66/S FT-IR spectrometer, UV spectra on a Shimadzu UV-1601 UV-Visible spectrophotometer, and ESI mass spectra on an Applied Biosystems Mariner time-of-flight mass spectrometer with an electrospray interface. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), with chemical shifts given in ppm (δ). Semi-preparative high-performance liquid chromatography (HPLC) was performed using a Gilson 306 pump with a Shodex refractive index detector. Silica gel 60 (Merck, 230-400 mesh) and RP-C_{18} silica gel (Merck, 230-400 mesh) were used for column chromatography (CC). The packing material for molecular sieve column chromatography was Sephadex LH-20. Merck precoated silica gel F_{254} and RP-18 F_{254s} plates were used for thin layer chromatography (TLC). Compounds were detected on TLC either under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Plant material: *E. alatus* twigs were collected from Chungju, Chungcheongbuk-do, Korea, in March 2010. Samples of plant material were identified by one of the authors (K.R. Lee). A voucher specimen (SKKU 2010-3) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation: The finely chopped plant material (5.2 kg) was extracted with 80% aqueous MeOH two times (each 20 L × 4 h) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a crude MeOH extract (220 g), which was suspended in distilled water (3.6 L) and then successively solvent-partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, yielding 31, 11, and 35 g of residue, respectively. Each fraction was evaluated for cytotoxic activity against human tumor cell lines, A549, SK-OV-3, and SK-MEL-2 using the SRB bioassay [17].

The $CHCl_3$-soluble fraction (11 g) was separated by silica gel (230-400 mesh) CC [500 g, 5 × 55 cm, eluted with $CHCl_3$-MeOH, 20:1 (1.5 L), 10:1 (1.5 L), and 1:1 (1.0 L)] to give 7 fractions (A1 – A7). Fractions A1, A2, and A3 were consolidated on the basis of TLC analysis, and the combined fraction (2.3 g) was applied to RP-C_{18} silica gel (230-400 mesh, 150 g) CC using a gradient solvent system of MeOH-H_2O (1:1 – 4:1 – 1:0) to give 16 sub-fractions (B1 – B16). Sub-fraction B1 (150 mg) was applied to a Sephadex LH-20 column using a solvent system of CH_2Cl_2-MeOH (1:1) to give 5 fractions (B1-1 – B1-5). Fraction B1-5 (45 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of MeOH-H_2O (97:3, 700 mL, flow rate; 2 mL/min) to yield compounds **4** (5 mg, t_R 16.5 min) and **11** (6 mg, t_R 23.0 min). Sub-fraction B9 (78 mg) was purified by semi-preparative normal-phase HPLC using a solvent system of $CHCl_3$-MeOH (45:1, 500 mL, flow rate; 2 mL/min) to give compounds **3** (13 mg, t_R 15.3 min) and **7** (5 mg, t_R 12.1 min). Sub-fraction B10 (40 mg) was also separated by HPLC purification with a solvent system of $CHCl_3$-MeOH (45:1, 500 mL, flow rate; 2 mL/min) to afford compound **6** (15 mg, t_R 13.0 min). Sub-fraction B12 (25 mg) was purified by semi-preparative normal-phase HPLC with a solvent system of $CHCl_3$-MeOH (40:1, 500 mL, flow rate; 2 mL/min) to obtain compound **8** (4 mg, t_R 11.5 min). Sub-fraction B13 (350 mg) was applied to a Sephadex LH-20 column with a solvent system of CH_2Cl_2-MeOH (1:1) to give 6 fractions (B13-1 – B13-6). Fractions B13-4 and B13-5 were consolidated, and the combined fraction (24 mg) was purified by semi-preparative normal-phase HPLC using *n*-hexane-EtOAc (4:1, 800 mL, flow rate; 2 mL/min) to yield compound **5** (5 mg, t_R 17.6 min). Sub-fraction B14 (300 mg) was applied to a Sephadex LH-20 column using CH_2Cl_2-MeOH (1:1) to furnish 5 fractions (B14-1 – B14-5). Fractions B14-3 and B14-4 were combined, and the combined fraction (47 mg) was purified by semi-preparative normal-phase HPLC using *n*-hexane-EtOAc (3:1, 800 mL, flow rate; 2 mL/min) to give compound **2** (10 mg, t_R 15.0 min). Fraction B15 (540 mg) was subjected to a Sephadex LH-20 column using CH_2Cl_2-MeOH (1:1) to obtain compound **1** (10 mg) and 3 fractions (B15-1 – B15-3). Fraction B15-2 (15 mg) was purified by semi-preparative reversed-phase HPLC using MeOH-H_2O (98:2, 700 mL, flow rate; 2 mL/min) to give compound **13** (8 mg, t_R 17.3 min). Fraction A4 (1.2 g) was applied to a Sephadex LH-20 column with a solvent system of CH_2Cl_2-MeOH (1:1) to give 5 sub-fractions (C1 – C5). Sub-fractions C3, C4, and C5 were consolidated, and the combined fraction (640 mg) was applied to silica gel (230-400 mesh, 80 g) CC using a solvent system of $CHCl_3$-MeOH (25:1) to yield 8 fractions (C3-1 – C3-8). Fractions C3-5, C3-6, and C3-7 were combined, and the combined fraction (65 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of MeOH-H_2O (93:7, 700 mL, flow rate; 2 mL/min) to afford compounds **9** (6 mg, t_R 17.5 min) and **14** (5 mg, t_R 18.3 min). Finally, fraction C3-8 (150 mg) was filtered with a solvent system of MeOH-H_2O (1:1), and the remaining residue (35 mg) was purified by HPLC with a solvent system of MeOH-H_2O (93:7, 700 mL, flow rate; 2 mL/min) to give compounds **10** (4 mg, t_R 15.0 min) and **12** (4 mg, t_R 18.5 min).

Supplementary data: Physico-chemical properties and ^{13}C NMR data of compounds **1-14** and bioassay protocol are available in electronic form on the publisher's website.

Acknowledgments - This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2A10005315).

References

[1] Mahato SB, Sarkar SK, Poddar G. (**1988**) Triterpenoid saponins. *Phytochemistry, **27***, 3037-3067.

[2] Sun H, Fang WS, Wang WZ, Hu C. (**2006**) Structure-activity relationships of oleanane- and ursane- type triterpenoids. *Botanical Studies*, ***47***, 339-368.
[3] Cassels BK, Asencio M. (**2011**) Anti-HIV activity of natural triterpenoids and hemisynthetic derivatives 2004-2009. *Phytochemistry Reviews*, ***10***, 545-564.
[4] Dzubak P, Hajduch M, Vydra D, Hustova A, Kvasnica M, Biedermann D, Markova L, Urban M, Sarek J. (**2006**) Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural Product Reports*, ***23***, 394-411.
[5] Patlolla JM, Rao CV. (**2012**) Triterpenoids for cancer prevention and treatment: current status and future prospects. *Current Pharmaceutical Biotechnology*, ***13***, 147-155.
[6] Petronelli A, Pannitteri G, Testa U. (**2009**) Triterpenoids as new promising anticancer drugs. *Anticancer Drugs*, ***20***, 880-892.
[7] Pack JH, Sung SH. (**2007**) *Medicinal plants.* Shinil Books Co., Seoul, 446-447.
[8] Park WH, Lee TK, Kim DI, Han JY, Kim CH. (**2004**) Inhibitory effects of *Scutellaria barbata* D. Don. and *Euonymus alatus* Sieb. on aromatase activity of human leiomyomal cells. *Immunopharmacology and Immunotoxicology*, ***26***, 315-327.
[9] Parka WH, Kim SH, Kim CH. (**2005**) A new matrix metalloproteinase-9 inhibitor 3,4-dihydroxycinnamic acid (caffeic acid) from methanol extract of *Euonymus alatus*: isolation and structure determination. *Toxicology*, ***207***, 383-390.
[10] Chung TW, Moon SK, Chang YC, Ko JH, Lee YC, Cho G, Kim SH, Kim JG, Kim CH. (**2004**) Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. *The FASEB Journal*, ***18***, 1670-1681.
[11] Ishiwata H, Shizuri Y, Yamada K. (**1983**) Three sesquiterpene alkaloids from *Euonymus alatus* forma *striatus*. *Phytochemistry*, ***22***, 2839-2841.
[12] Fang XK, Gao J, Zhu DN. (**2008**) Kaempferol and quercetin isolated from *Euonymus alatus* improve glucose uptake of 3T3-L1 cells without adipogenesis activity. *Life Sciences*, ***82***, 615-622.
[13] Jeong EJ, Yang H, Kim SH, Kang SY, Sung SH, Kim YC. (**2011**) Inhibitory constituents of *Euonymus alatus* leaves and twigs on nitric oxide production in BV2 microglia cells. *Food and Chemical Toxicology*, ***49***, 1394-1398.
[14] Yan ZH, Han ZZ, Hu XQ, Liu QX, Zhang WD, Liu RH, Li HL. (**2013**) Two new sesquiterpenes from *Euonymus alatus*. *Helvetica Chimica Acta*, ***96***, 85-92.
[15] Jeong EJ, Bae JY, Rho JR, Kim YC, Ahn MJ, Sung SH. (**2014**) Anti-inflammatory triterpenes from *Euonymus alatus* leaves and twigs on lipopolysaccharide-activated RAW264.7 macrophage cells. *Bulletin of the Korean Chemical Society*, ***35***, 2945-2949.
[16] Kim KH, Ha SK, Choi SU, Kim SY, Lee KR. (**2013**) Phenolic constituents from the twigs of *Euonymus alatus* and their cytotoxic and anti-inflammatory activity. *Planta Medica*, ***79***, 361-364.
[17] Song JH, Choi HJ, Song HH, Hong EH, Lee BR, Oh SR, Choi K, Yeo SG, Lee YP, Cho S, Ko HJ. (**2014**) Antiviral activity of ginsenosides against coxsackievirus B3, enterovirus 71, and human rhinovirus 3. *Journal of Ginseng Research*, ***38***, 173-179.
[18] Reynolds WF, McLean S, Poplawski J, Enriquez RG, Escobar LI, Leon I. (**1986**) Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivatives by 2D NMR: an investigation of the potential utility of ^{1}H chemical shifts in structural investigations of complex natural products. *Tetrahedron*, ***42***, 3419-3428.
[19] Tinto WF, Blair LC, Alli A, Reynolds WF, McLean S. (**1992**) Lupane triterpenoids of *Salacia cordata*. *Journal of Natural Products*, ***55***, 395-398.
[20] González AG, Jiménez IA, Ravelo AG. (**1992**) Triterpenes from *Maytenus canariensis* and synthesis of a derivative from betulin. *Phytochemistry*, ***31***, 2069-2072.
[21] Puapairoj P, Naengchomnong W, Kijjoa A, Pinto MM, Pedro M, Nascimento MSJ, Silva AMS, Herz W. (**2005**) Cytotoxic activity of lupane-type triterpenes from *Glochidion sphaerogynum* and *Glochidion eriocarpum* two of which induce apoptosis. *Planta Medica*, ***71***, 208-213.
[22] Hata K, Hori K, Takahashi S. (**2002**) Differentiation- and apoptosis-inducing activities by pentacyclic triterpenes on a mouse melanoma cell line. *Journal of Natural Products*, ***65***, 645-648.
[23] Satiraphan M, Pamonsinlapatham P, Sotanaphun U, Sittisombut C, Raynaud F, Garbay C, Michel S, Cachet X. (**2012**) Lupane triterpenes from the leaves of the tropical rain forest tree *Hopea odorata* Roxb. and their cytotoxic activities. *Biochemical Systematics and Ecology*, ***44***, 407-412.
[24] Macías FA, Simonet AM, Esteban MD. (**1994**) Potential allelopathic lupane triterpenes from bioactive fractions of *Melilotus messanensis*. *Phytochemistry,* ***36***, 1369-1379.
[25] El-Seedi HR. (**2005**) Antimicrobial triterpenes from *Poulsenia armata* (Miq.) Standl. *Natural Product Research*, ***19***, 197-202.
[26] Taniguchi S, Imayoshi Y, Kobayashi E, Takamatsu Y, Ito H, Hatano T, Sakagami H, Tokuda H, Nishino H, Sugita D, Shimura S, Yoshida T. (**2002**) Production of bioactive triterpenes by *Eriobotrya japonica* calli. *Phytochemistry*, ***59***, 315–323.
[27] Kizu H, Tomimori T. (**1982**) Studies on the constituents of *Clematis* species. V. On the saponins of the root of *Clematis chinensis* OSBECK. (5). *Chemical and Pharmaceutical Bulletin*, ***30***, 3340–3346.
[28] de Almeida MTR, Ríos-Luci C, Padrón JM, Palermo JA. (**2010**) Antiproliferative terpenoids and alkaloids from the roots of *Maytenus vitis-idaea* and *Maytenus spinosa*. *Phytochemistry*, ***71***, 1741-1748.
[29] Okada Y, Omae A, Okuyama T. (**2003**) A new triterpenoid isolated from *Lagerstronemia speciosa* (L.) Pers. *Chemical and Pharmaceutical Bulletin*, ***51***, 452–454.
[30] Seebacher W, Simic N, Weis R, Saf R, Kunert O. (**2003**) Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. *Magnetic Resonance in Chemistry*, ***41***, 636–638.
[31] Beekman AC, Woerdenbag HJ, van Uden W, Pras N, Konings AWT. (**1997**) Structure-cytotoxicity relationships of some helenanolide-type sesquiterpene lactones. *Journal of Natural Products*, ***60***, 252-257.

Natural Product Communications Vol. 10 (11) 2015
Published online (www.naturalproduct.us)

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Natural Product Communications
2015
Volume 10, Number 11

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