# Antitumor Activity of *Pulsatilla koreana* Saponins and Their Structure–Activity Relationship

Seong-Cheol BANG,[a,b] Ji-Hyun LEE,[a] Gyu-Yong SONG,[b] Dong-Hee KIM,[c] Mi-Young YOON,[c] and Byung-Zun AHN*,[a]

[a] *SB Pharmaceutical Co.; Gongju 314–923, Chungnam, Korea:* [b] *College of Pharmacy, Chungnam National University; Daejeon 305–764, Korea: and* [c] *Department of Pathology, College of Oriental Medicine, Daejeon University; Daejeon 300–716, Korea.* Received January 27, 2005; accepted August 1, 2005

**Seventeen saponins isolated from the root of *Pulsatilla koreana* were examined for their *in vitro* cytotoxic activity against the human solid cancer cell lines, A-549, SK-OV-3, SK-MEL-2, and HCT15, using the SRB assay method, and their *in vivo* antitumor activity using BDF1 mice bearing Lewis lung carcinoma (LLC). The saponins 5—17, with a free acidic functional group at C-28 of aglycon, exhibited moderate to considerable cytotoxic activity, however, the saponins 1—4, esterified with a trisaccharide at C-28 of aglycon, did not exhibit cytotoxic activity ($ED_{50}$; >300 $\mu$M). Among them, oleanolic acid 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside (10) exhibited the most potent cytotoxic activity ($ED_{50}$; 2.56, 2.31, 1.57, 8.36 $\mu$M, respectively). *In vivo* test, hederagenin 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside (6, Inhibition Ratio, IR; 66.9%) exhibited more potent antitumor activity than taxol (IR; 35.8%) and doxorubicin (IR; 62.1%). Also, hedragenin 3-*O*-$\beta$-D-glucopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (17, IR; 50.3%) exhibited potent antitumor activity. These two saponins were identically comprised of a hederagenin aglycon moiety and a sugar sequence *O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside at C-3 of the hederagenin, suggesting that the two elements are essential factors for the antitumor activity.**

**Key words** *Pulsatilla koreana* saponin; cytotoxicity; antitumor activity

*Pulsatilla koreana* N. belongs to the family Ranunculaceae and is an endemic species in Korea. The roots of this plant have been widely used in traditional medicine for the treatment of several diseases, particular malaria and amoebic dysentery.[1] This plant has been investigated extensively, resulting in the isolation of various saponins, ranunculin, anemonin, protoanemonin and triterpenes.[2—4] Our continuing investigation of the chemical constituents of the *P. koreana* roots has resulted in the discovery of many saponins (Fig. 1).[5] However, to the best of the author's knowledge, there is no report on the systematic cytotoxic and antitumor activity of the saponins isolated from the roots of this plant. The variety of their structures motivated us to examine the structure–activity relationship with the *in vitro* cytotoxic activity as well as the *in vivo* antitumor activity.

17 saponins, eight lupane-type (**1**, **3**, **5**, **7**, **9**, **11**, **13**, **15**) and nine oleanane-type (**2**, **4**, **6**, **8**, **10**, **12**, **14**, **16**, **17**), isolated from *P. koreana* roots, were evaluated for their cytotoxic activity against four human solid tumor cell lines (A-549, SK-OV-3, SK-MEL-2, HCT-15). So far cytotoxic activities against tumor cell lines of these saponins have not been reported. $ED_{50}$ values obtained were reported in Table 1. As shown in Table 1, it was observed that the saponins **5**—**17**, possessed free carboxylic group at C-28, exhibited moderate to considerable cytotoxic activity ($ED_{50}$; 1.57—174.34 $\mu$M) against tumor lines, whereas their sugar-bonded esters, that is, disdesmoside saponins **1**, **2**, **3**, and **4** were inactive ($ED_{50}$ >300 $\mu$M). From this result, we postulate the existence of a free carboxylic group at C-28 is important for the cytotoxic activity. Among the cytotoxic saponins **5**—**17**, oleanolic acid 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside (**10**) and oleanolic acid 3-*O*-$\beta$-D-glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside (**14**) exhibited the strongest cytotoxic activity ($ED_{50}$; 1.57—8.36 $\mu$M and 3.47—5.50 $\mu$M, respectively), while saponin **6** and **8**, possessed hederagenin as an aglycon, exhibited weaker cytotoxic activity. From these results, we postulate the hydroxyl group at C-23 had a negative effect on the cytotoxic activity. This observation bears a striking resemble to another recent study, conducted by Barthomeuf *et al.*[6] This may be attributed to the electron donating effect of two lone-paired electrons of hydroxyl group toward C-3 of the aglycon (Fig. 2).[7]

In a study to determine the relationship between the structure of the sugar moiety at C-3 and the cytotoxic activity, saponins **5** and **6** possessing a trisaccharide of 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-*O*-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranosyl group, were shown to have a better cytotoxic activity than saponins **15** and **16** which had a disaccharide of the 3-*O*-$\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-arabinopyranosyl group. This means that the presence of rhamnopyranose linked at C-2′ of the arabinopyranosyl group enhances the cytotoxic activity of monodesmosides. In addition, it was found that there are differences in cytotoxicity between oleanane and lupane saponins. Generally, the cytotoxicities ($ED_{50}$; 38.14—176.34 $\mu$M) of the lupine-type saponins were much weaker than those ($ED_{50}$; 1.57—13.58 $\mu$M) of the oleanane-type saponins, and this result was in agreement with those reported before for similar compounds.[8] Finally, the cell specific action of the saponins was observed. Although the saponins showed no great difference in the cytotoxicity, saponins **6** and **10** exhibited a stronger cytotoxicity with $ED_{50}$ of 1.57 and 3.04 $\mu$M against the SK-MEL-2 cell line, respectively.

In order to investigate their antitumor activity, among the 17 saponins isolated from *P. koreana* roots, 15 saponins which could be isolated in sufficient quantities were applied for the animal test. During the period, there was no remark-

* To whom correspondence should be addressed. e-mail: ahnbj@cnu.ac.kr
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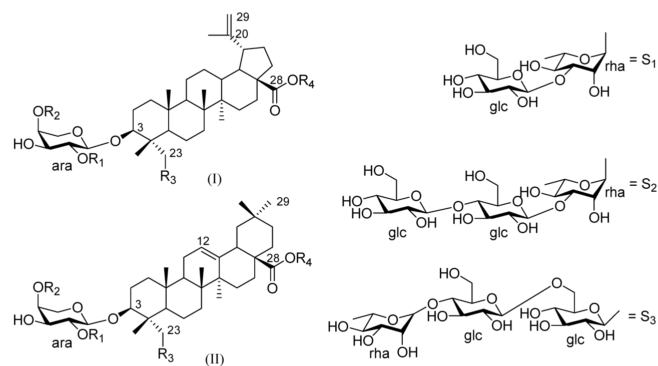


| Saponin | aglycon[a] | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| **1** | (I) | rha | glc | OH | $S_3$ |
| **2** | (II) | rha | glc | OH | $S_3$ |
| **3** | (I) | rha | H | OH | $S_3$ |
| **4** | (II) | rha | H | OH | $S_3$ |
| **5** | (I) | rha | glc | OH | H |
| **6** | (II) | rha | glc | OH | H |
| **7** | (I) | $S_1$ | H | OH | H |
| **8** | (II) | $S_1$ | H | OH | H |
| **9** | (I) | rha | glc | H | H |
| **10** | (II) | rha | glc | H | H |
| **11** | (I) | rha | H | OH | H |
| **12** | (II) | rha | H | OH | H |
| **13** | (I) | $S_1$ | H | H | H |
| **14** | (II) | $S_1$ | H | H | H |
| **15** | (I) | H | glc | OH | H |
| **16** | (II) | H | glc | OH | H |
| **17** | (II) | $S_2$ | H | OH | H |

*a*) Lupane-type saponins expressed as (I) and oleanane-type saponins as (II).

Fig. 1. The Structures of Saponins Isolated from *P. koreana* Roots

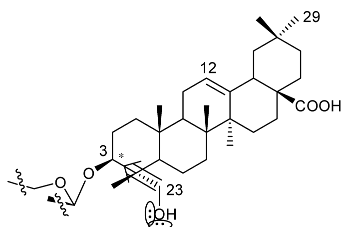


Fig. 2. The Electron Donating Effect of Hydroxyl Group Attached at C-23 Has a Decreasing Effect on the Cytotoxic Activity of Saponins

able weight loss or evidence of toxicity by macroscopic examination of the organ in non-tumors-bearing mice treated with each samples (not shown). The saponins and their antitumor activity were displayed in Table 2. First of all, we detected that saponins **2**, **3**, and **4**, which contain an ester group at C-28 of aglycon, were inactive. With the exception of saponin **1**, this result was coincident with the cytotoxic activity mentioned above. The straight-linked trisaccharide moiety at C-28 appeared to interrupt the activity. Among the tested saponins with a free carboxylic acid group at C-28, an oleanane-type saponin **6** had the most potent antitumor activity (IR; 66.9%), and saponin **14** (IR; 48.8%) and **17** (IR; 50.3%) also showed a good antitumor activity. On the 17th day after tumor transplantation, four tumor masses extirpated from mice which were tested by these saponins was displayed with control in Fig. 2. All of them were more potent in the *in vivo* activity than Taxol® (IR; 35.8%) used as a positive control. There was not obvious co-relationship between the *in vitro* cytotoxicity and the *in vivo* antitumor activity. For example, it is very interesting to find that saponin **10** (IR;

Table 1. Cytotoxic Activity of Isolated *Pusatilla* Saponins against Four Human Cancer Cell Lines

| Saponins | $ED_{50}$ ($\mu$M)[a] | | | |
|---|---|---|---|---|
| | A-549 | SK-OV-3 | SK-MEL-2 | HCT15 |
| **1** | >300.0 | >300.0 | >300.0 | >300.0 |
| **2** | >300.0 | >300.0 | >300.0 | >300.0 |
| **3** | >300.0 | >300.0 | >300.0 | >300.0 |
| **4** | >300.0 | >300.0 | >300.0 | >300.0 |
| **5** | 38.14 | 36.27 | 37.44 | 40.06 |
| **6** | 11.25 | 13.17 | 3.04 | 11.86 |
| **7** | 145.62 | 120.38 | 167.82 | 174.34 |
| **8** | 13.27 | 11.41 | 12.16 | 13.58 |
| **9** | 135.28 | 114.32 | 165.54 | 171.29 |
| **10** | 2.56 | 2.31 | 1.57 | 8.36 |
| **11** | 43.64 | 38.90 | 39.67 | 49.04 |
| **12** | 13.49 | 13.71 | 14.12 | 14.17 |
| **13** | 155.32 | 124.12 | 145.68 | 138.73 |
| **14** | 4.24 | 3.95 | 3.47 | 5.50 |
| **15** | 41.35 | 38.91 | 40.02 | 40.86 |
| **16** | 9.58 | 11.39 | 10.37 | 12.74 |
| **17** | 10.73 | 10.66 | 9.81 | 14.09 |
| Doxorubicin[b] | 0.017 | 0.094 | 0.036 | 0.792 |

*a*) $ED_{50}$ value was defined as the concentration of compounds needed to reduce a 50% of absorbance relative to the vehicle-treated controls. The data are mean values of three experiments performed in triplicate. *b*) Doxorubicin as positive control.

37.8%) having oleanolic acid as an aglycon, which showed the strongest cytotoxic activity, had an inferior *in vivo* antitumor activity than saponin **6** having hederagenin. It was supposed that the hederagenin structure of **6** might enhance more bioavailability to modulate cell permeablization *via* complexation of sterol in the plasma membrane than the oleanolic acid of **10** in the mouse tumor tissue.[9] As for the sugar moiety, saponin **6** having $\alpha$-L-rhamnopyranosyl-(1→2)-[*O*-$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside at C-3 of aglycon had a more potent activity than saponin **8** (IR; 28.0%) which had $\alpha$-D-glucopyranosyl-(1→3)-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranose at the same position. Also, in case of saponin possessing straight-linked trisaccharide chain, the addition of a glucopyranoside enhanced the antitumor activity. For instance, saponin **17**, produced by attaching one more glucopyranoside to C-4‴ of glucopyranosyl moiety in saponin **8**, exhibited a significant increase of 22% in the antitumor activity. In contrast, saponins **12** or **16** due to eliminating a glucopyranoside from saponin **6** reduced the antitumor activity to 20.1 and 22.8%, respectively. These results indicate that both the binding position and the number of glucopyranoside linked with the sugar sequence *O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside of saponin **12** ($\alpha$-hederin) play an important role in the antitumor activity. This can be explained by the rate of glucose hydrolysis in the cell. Presumably, the C-4″ position of $\alpha$-hederin than C-2″ may be insensitive to glucose-hydrolase. Therefore the probability of reaching the active saponin to targeting site increases, suggesting that the activity is elevated. By the same token, increase in number of sugar increase the activity by declining the rate of hydrolysis, too. With regard to aglycon, saponin **5** having 23-hydroxybetulinic acid as the aglycon and the same sugar moiety as **6** had a lower antitumor activity (IR; 27.5%). The same phenomenon was observed for the pair of saponins **7** (no activity) and **8** as well as saponins **9** (IR; 6.3%) and **10**. It was

Table 2. Antitumor Activity of Isolated *Pulsatilla* Saponins on BDF1 Bearing LLC (%, Inhibition Ratio)

| Saponins | Chemical formular | MW[b] (molecular weight) | Treatment dose[c] | | Antitumor activity[a] T.V., $mm^3 \pm$S.D. (I.R., %) |
|---|---|---|---|---|---|
| | | | $\mu$mol/kg/d | mg/kg/d | |
| **1** | $C_{65}H_{106}O_{31}$ | 1382 | 6.6 | 9.1 | 1484.1±642.4 (35.3) |
| **2** | $C_{65}H_{106}O_{31}$ | 1382 | 6.6 | 9.1 | NA[d] |
| **3** | $C_{59}H_{96}O_{26}$ | 1220 | 6.6 | 8.0 | NA |
| **4** | $C_{59}H_{96}O_{26}$ | 1220 | 6.6 | 8.0 | NA |
| **5** | $C_{47}H_{76}O_{17}$ | 912 | 6.6 | 6.0 | 1663.0±283.5 (27.5) |
| **6** | $C_{47}H_{76}O_{17}$ | 912 | 6.6 | 6.0 | 759.2±327.7 (66.9) |
| **7** | $C_{47}H_{76}O_{17}$ | 912 | 6.6 | 6.0 | NA |
| **8** | $C_{47}H_{76}O_{17}$ | 912 | 6.6 | 6.0 | 1651.5±212.4 (28.0) |
| **9** | $C_{47}H_{76}O_{16}$ | 896 | 6.6 | 5.9 | 2149.3±245.4 (6.3) |
| **10** | $C_{47}H_{76}O_{16}$ | 896 | 6.6 | 5.9 | 1426.7±575.6 (37.8) |
| **11** | $C_{41}H_{66}O_{12}$ | 750 | 6.6 | 4.9 | 1493.3±363.5 (34.9) |
| **12** | $C_{41}H_{66}O_{12}$ | 750 | 6.6 | 4.9 | 1832.7±441.3 (20.1) |
| **13** | $C_{47}H_{76}O_{16}$ | 896 | 6.6 | 5.9 | NT[e] |
| **14** | $C_{47}H_{76}O_{16}$ | 896 | 6.6 | 5.9 | 1174.4±334.6 (48.8) |
| **15** | $C_{41}H_{66}O_{13}$ | 766 | 6.6 | 5.0 | NT |
| **16** | $C_{41}H_{66}O_{13}$ | 766 | 6.6 | 5.0 | 1770.8±453.7 (22.8) |
| **17** | $C_{53}H_{86}O_{22}$ | 1074 | 6.6 | 7.2 | 1140.0±224.6 (50.3) |
| Taxol®[f] | — | — | — | 12.5 | 1472.6±472.8 (35.8) |
| Doxorubicin[g] | — | — | — | 1.0 | 869.4±486.7 (62.1) |
| Control | — | — | — | — | 2293.8±343.6 |

*a*) The antitumor activity was determined as tumor volume (T.V.) as mean value ($mm^3$)±S.D. and inhibition rate (I.R.) as % in parentheses, in comparison with untreated control group as described on experimental section and each sample was injected at a maximum tolerate dose of 6.6 $\mu$mol/kg/d, respectively. *b*) Molecular weight was determined by FAB-mass spectra on a JMS-HX110/110A spectrometer. *c*) Each group consists of five BDF1 mice bearing Lewis lung carcinoma (LLC). *d*) No activity. *e*) No test. *f*, *g*) Taxol® and doxorubicin were used as positive control at dose of 12.5 mg/kg/d and 1.0 mg/kg/d, respectively.

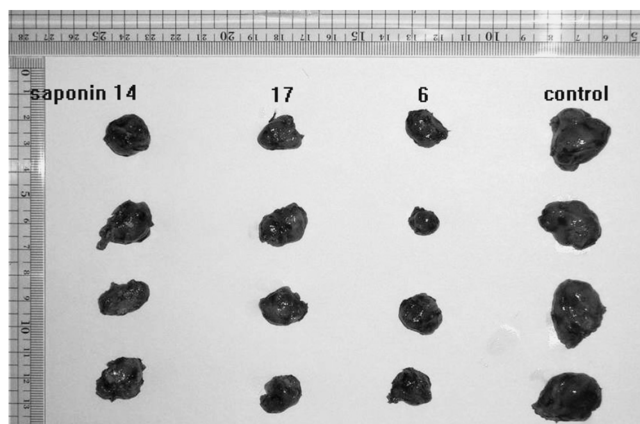


Fig. 3. Comparison of Four Tumor Masses Extirpated from Mice Tested by Three Saponins **6**, **14**, and **17** with Control on the 17th Day after Tumor Transplantation

concluded that oleanane-type saponins showed more potent than the lupane-type saponins *in vivo* as well as *in vitro*. In conclusion, this study gave essential information of structure–activity relationship of *Pulsatilla* saponins for the *in vitro* and *in vivo* activities. We found that the characteristic structural features of potent antitumor saponins were to have $\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl group at C-3 and a free carboxylic acid at C-28 of the oleanane skeleton. At this time, the presence of hydroxyl group at C-23 of aglycon, of the third glucopyranoside attached at C-4″ of sugar moiety, and the addition of the fourth sugar made the antitumor activity more effective *in vivo*.

## Experimental

**General Procedures** Melting points were measured on an Electrothermal melting point apparatus. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. FAB-MS were recorded on a JMS-HX110/110A spectrometer. The following materials and reagents were used for cell culture and the sulforhodamine B (SRB) assay: microplate reader, Tecan A-5082 (Salzburg, Austria); 96-well flat-bottom plate, Falcon (Bedford, MA, U.S.A.); RPMI-1640 medium and FBS, Gibco BRL (Rockville, MD, U.S.A.). All other chemicals used were of biochemical reagent grade.

**Plant Material** The roots of *Pulsatilla koreana* were collected at Geumsan-gun, Chungnam, Korea in May 2003 and authenticated by Professor Bae, Chungnam National University. A voucher specimen was deposited in the herbarium of Chungnam College of Pharmacy.

**Extraction and Isolation** The saponins **1**—**17** used in this study were isolated from *P. koreana* roots as the same method described previously.[5)]

Saponin **1**: 23-Hydroxy-3$\beta$-[(*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-oic acid 28-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester: White amorphous powder, mp 216—218 °C, $[\alpha]_D^{26}$ −38.4° ($c$=0.1, MeOH). FAB-MS *m/z*: 1405 $[M+Na]^+$. HR-FAB-MS (positive mode) *m/z*: 1406.5242 (Calcd for $C_{65}H_{106}O_{31}Na$, 1406.5278).

Saponin **2**: 3-*O*-$\alpha$-L-Rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranosyl hederagenin 28-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester: White amorphous powder, mp 205—208 °C, $[\alpha]_D^{21}$ −17.1° ($c$=0.36, MeOH). FAB-MS *m/z*: 1405 $[M+Na]^+$. HR-FAB-MS (positive mode) *m/z*: 1406.5218 (Calcd for $C_{65}H_{106}O_{31}Na$, 1406.5278).

Saponin **3**: 23-Hydroxy-3$\beta$-[(*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-oic acid 28-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-*O*-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester: White amorphous powder, mp 208—215 °C, $[\alpha]_D^{21}$ −34.9° ($c$=1.13, MeOH). FAB-MS *m/z*: 1243 $[M+Na]^+$. HR-FAB-MS (positive mode) *m/z*: 1244.3792 (Calcd for $C_{59}H_{96}O_{26}Na$, 1244.3854).

Saponin **4**: 3-*O*-$\alpha$-L-Rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl hederagenin 28-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl ester: White amorphous powder, mp 225—228 °C, $[\alpha]_D^{20}$ −13.5° ($c$=0.3, MeOH). FAB-MS *m/z*: 1243 $[M+Na]^+$. HR-FAB-MS (positive mode) *m/z*: 1244.3837 (Calcd for $C_{59}H_{96}O_{26}Na$, 1244.3854).

Saponin **5**: 23-Hydroxy-3$\beta$-[(*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid: White amorphous powder, mp 250—252 °C, $[\alpha]_D^{25}$ −11.3° ($c$=0.1, MeOH). FAB-MS *m/z*: 935 $[M+Na]^+$. HR-FAB-MS (positive mode) *m/z*: 936.1103 (Calcd for $C_{47}H_{76}O_{17}Na$, 936.1071).

Saponin **6**: Hederagenin 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside: White amorphous powder, mp

239—241 °C, $[\alpha]_D^{25}$ +23.6° ($c$=0.2, MeOH). FAB-MS $m/z$: 935 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 936.1042 (Calcd for $C_{47}H_{76}O_{17}Na$, 936.1071).

Saponin **7**: 23-Hydroxy-3$\beta$-[($O$-$\beta$-D-glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid: White amorphous powder, mp 252—254 °C, $[\alpha]_D^{25}$ −11.3° ($c$=0.1, MeOH). FAB-MS $m/z$: 935 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 936.1137 (Calcd for $C_{47}H_{76}O_{17}Na$, 936.1071).

Saponin **8**: Hederagenin 3-$O$-$\beta$-D-glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 240—245 °C, $[\alpha]_D^{22}$ +7.6° ($c$=1.4, pyridine). FAB-MS $m/z$: 935 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 936.1102 (Calcd for $C_{47}H_{76}O_{17}Na$, 936.1071).

Saponin **9**: 3$\beta$-[($O$-$\alpha$-L-Rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid: White amorphous powder, mp 247—250 °C, $[\alpha]_D^{25}$ −5.9° ($c$=0.05, MeOH). FAB-MS $m/z$: 919 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 920.1061 (Calcd for $C_{47}H_{76}O_{16}Na$, 920.1077).

Saponin **10**: Oleanolic acid 3-$O$-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\beta$-D-glucopyranosyl-(1→4)]-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 248—251 °C, $[\alpha]_D^{25}$ −6.11° ($c$=0.14, EtOH). FAB-MS $m/z$: 919 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 920.1053 (Calcd for $C_{47}H_{76}O_{16}Na$, 920.1077).

Saponin **11**: 23-Hydroxy-3$\beta$-[($O$-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-oic acid: White amorphous powder, mp 228—230 °C, $[\alpha]_D^{25}$ −7.8° ($c$=0.56, MeOH). FAB-MS $m/z$: 773 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 773.9536 (Calcd for $C_{41}H_{66}O_{12}Na$, 773.9576).

Saponin **12**: Hederagenin 3-$O$-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 264—265 °C, $[\alpha]_D^{25}$ +16.9° ($c$=0.13, MeOH). FAB-MS $m/z$: 773 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 773.9598 (Calcd for $C_{41}H_{66}O_{12}Na$, 773.9576).

Saponin **13**: 3$\beta$-[($O$-$\beta$-D-Glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid: White amorphous powder, mp 245—250 °C, $[\alpha]_D^{25}$ −6.0° ($c$=0.05, MeOH). FAB-MS $m/z$: 919 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 920.1094 (Calcd for $C_{47}H_{76}O_{16}Na$, 920.1077).

Saponin **14**: Oleanolic acid 3-$O$-$\beta$-D-glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 245—250 °C, $[\alpha]_D^{25}$ −2.5° ($c$=0.1, MeOH). FAB-MS $m/z$: 919 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 920.1048 (Calcd for $C_{47}H_{76}O_{16}Na$, 920.1077).

Saponin **15**: 23-Hydroxy-3$\beta$-[($O$-$\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid: White amorphous powder, mp 267—269 °C, $[\alpha]_D^{25}$ −27.7° ($c$=0.09, MeOH). FAB-MS $m/z$: 789 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 789.9627 (Calcd for $C_{41}H_{66}O_{13}Na$, 789.9632).

Saponin **16**: Hederagenin 3-$O$-$\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 242—244 °C, $[\alpha]_D^{25}$ +49.8° ($c$=1.12 MeOH). FAB-MS $m/z$: 789 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 789.9615 (Calcd for $C_{41}H_{66}O_{13}Na$, 789.9632).

Saponin **17**: Hederagenin 3-$O$-$\beta$-D-glucopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinopyranoside: White amorphous powder, mp 260—262 °C, $[\alpha]_D^{25}$ −6.0° ($c$=0.1, MeOH). FAB-MS $m/z$: 1097 $[M+Na]^+$. HR-FAB-MS (positive mode) $m/z$: 1098.2516 (Calcd for $C_{53}H_{86}O_{22}Na$, 1098.2504).

**Tumor Cells** The human solid tumor cell lines, A-549, SK-OV-3, SK-MEL-2 and HCT15 cells, were purchased from KCLB (Korea Cell Line Bank, Seoul, Korea). The Lewis lung carcinoma (LLC) cells were obtained from the KRIBB (Korea Research Institute of Bioscience and Biotechnology).

**Animals** Male BDF1 mice, 20±2 g body weight, were purchased from SLC, Inc. (Japan), and fed commercial solid food (Samyang Yuji Co. Ltd., Seoul) and tap water. They were housed at 23±0.5 °C at 60% humidity in a 12 h light–dark cycle in accordance with the guidelines for the vare and use of Laboratory Animals by Chungnam National University.

***In Vitro* Cytotoxicity Assay** The cytotoxicity assay was carried out according to the SRB assay described previously.[10] Four human cancer cell lines, A-549 (Lung cancer), SK-OV-3 (Ovarian cancer), SK-MEL-2 (Skin cancer) and HCT-15 (Colon cancer), were examined. Doxorubicin was used as the positive control. Growth inhibition of 50% ($ED_{50}$) of the saponins was calculated using the method described elsewhere.[11]

***In Vivo* Antitumor Assay** The antitumor assay on the BDF1 mice bearing Lewis lung carcinoma (LLC) cells was carried out according to the procedures described previously.[12] Each sample were then dissolved in physiological saline, and injected intraperitoneally on the 1st to 4th, 6th to 9th, and 11th to 14th day at a maximum tolerate dose of 6.6 $\mu$mol/kg, respectively. The experimental mice were weighed every day in order to evaluate the toxicity of each sample. The tumor volume (T.V.) was measured three times on the 15th and 16th day and calculated according to the following formula and taxol® and doxorubicin were used as positive controls.[13,14]

$$\text{T.V. (tumor volume, mm}^3) = \frac{L\,(\text{mm}) \times W^2\,(\text{mm}^2)}{2}$$

where $L$ and $W$ represent the major axis and the width of the tumor mass, respectively. The inhibition ratio (I.R.) was calculated according to the following formula:

$$\text{I.R. (inhibition ratio, \%)} = \frac{(\text{mean T.V. of control group} - \text{mean T.V. of treated group})}{\text{mean T.V. of control group}} \times 100$$

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