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Isolation of ursane-type triterpene saponins from *Allium ascalonicum* L.

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***Abstract*—Six ursane-type triterpene saponins, including ilexkudinoside T (1), kudinoside D (2), randiasaponin IV (3), kudinoside G (4), ilexkudinoside W (5), lactifoloside G (6) were isolated from the rhizomes of *Allium ascalonicum* L. Their structures were determined by HR-ESI-MS, ^{1}D-NMR, and ^{2}D-NMR spectral data, and comparison with those reported in the literature. Compounds 1 and 2 possessed 20,28-lactone linkage which was unusual ursane-type triterpene saponins.**

Keywords*— *Shallot; Allium ascalonicum; Amaryllidaceae; Ursane-type triterpene; Structural elucidation.

I. INTRODUCTION

Shallot (*Allium ascalonicum* L., Alliaceae) has been used worldwide to be a vegetable and culinary supplements for along times [1]. In traditional medicinial remedies, *A. ascalonicum* has many benefits such as anti-inflammation, anti-oxidant, antibacterial properties [2, 3]. Aqueous extract of *A. ascalonicum* showed much less cytotoxic effect against normal cell but significant anti-growth activity on cancer cell and significant anti-inflammatory activity in the *in vitro* and *in vivo* studies [3-5]. Phytochemical study on *A. ascalonicum*, therefore, has been attractive scientists to find potential biological active components and clarify its therapeutic effects. Previous reports indicate that *A. ascalonicum* is a rich source of phenolics, saponins, and also sulfur containing compounds as general constituents in the *Allium* genus [6-8]. Contribution to clarify chemical constituents of *A. ascalonicum* and provide insightful explanation on its beneficial effects, herein, we report the isolation and structural elucidation of six ursane-type saponins from methanolic extract of *A. ascalonicum*. Saponins have been reviewed to be potential anti-inflammation and cytotoxic activity. Therefore, the identification of saponins could be helpful for explaintion of cytotoxic and anti-inflammatory activities of *A. ascalonicum* and their processed products.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were recorded on a Bruker AVANCE III 500 MHz (Bruker BioSpin, Bremen, Germany). HR-ESI-MS was accquired on an Agilent 6530 Accurate Mass QTOF LC/MS system (Agilent technology, Santa Clara, CA, USA). Column chromatography was carried out using silica gel (Merck, Whitehouse Station, NJ, USA), reversed phase C-18 gel (YMC Ltd., Kyoto, Japan), and diaion HP-20 resin as stationary phase. Thin layer chromatography was performed using pre-coated silica gel 60 F_{254} and RP-C18 F_{254S} plates (Merck, Darmstadt, Germany). Compounds were detected by spraying with aqueous solution of H_2SO_4 (5%, w/w) followed by heating on a hot plate.

B. Plant materials

The rhizomes of *Allium ascalonicum* L. were collected at Hanoi City, Vietnam, in December 2018. Its scientific name was determined by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Hanoi, Vietnam. Voucher specimen (NCCT.2019.78) is kept at the Institute of Marine Biochemistry, Hanoi, Vietnam.

C. Extraction and isolation

The dried powder of the rhizomes of *A. ascalonicum* (5.0 kg) was ultrasonically extracted with methanol for three times (10 L, each) to give methanol extract. This extract (340 g) was suspended with distilled water (4.0 L) and separated with dichloromethane to give dichloromethane faction and water layer. The water layer was loaded on a Diaion HP-20 column, washed with water, and then eluted with methanol/ water (1:3, 1:1, 3:1, and 1:0) to give four fractions, WA-WD. Fraction WB (38.0 g) was subjected on a silica gel column and eluted with CH_2Cl_2/MeOH (stepwise, 20:1, 10:1, 5:1, 2.5:1, 1:1) to give five fractions, WB1-WB5. Fraction WB3 (16.0 g) was chromatographed on a RP-18 column and eluted with acetone/water (1:1.2) to give four fractions, WB3A-WB3D. Fraction WB3B (3.5 g) was separated on a silica gel column and eluted with CH_2Cl_2/acetone/water (1:4:0.2) to give two fractions, WB3B1 and WB3B2. Compound **3** (3.6 mg) was isolated from fraction WB3B1 by HPLC using aqueous ACN (33%). Compounds **1** (9.1 mg) and **2** (15.6 mg) were purified from the WB3B2 fraction by HPLC using aqueous ACN (40%). Fraction WB3C was subjected on a silica gel column and eluted with CH_2Cl_2/acetone/water (1:4:0.3) to give three fractions, WB3C1-WB3C3. The WB3C1 fraction was purified by HPLC using aqueous ACN (30%) to give **5** (3.9 mg). Fraction WB (48 g) was loaded on a silica gel column

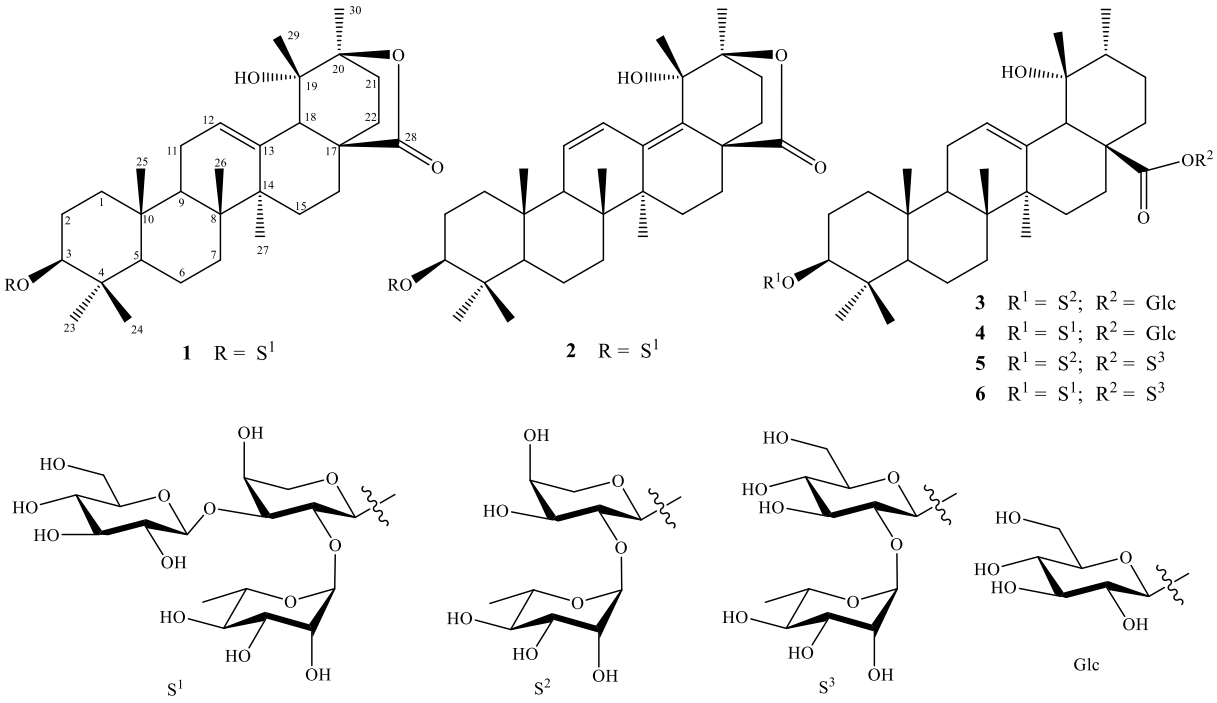

Fig. 1. *Structure of Ursane-type saponins **1**-6 isolated from Allium ascalonicum*

and eluted with CH_2Cl_2/MeOH (stepsiwe, 20:1, 5:1, 1:1) to give three fractions, WB1- WB3. Fraction WB1 (14.0 g) was separated on a RP-18 column eluting with acetone/water (5:6) to give four fractions, WB1A-WB1D. Fraction WB1B was chromatographed on a RP-18 column, eluting with acetone/water (4:1) and further purified by HPLC with aqueous acetonitrile (30%) to give **4** (6.4 mg). The WB2 fraction (15.0 g) was separated on a RP-18 column eluting with acetone/water (5:6) to give five fractions, WB2A-WB2E. Fraction WB2B (3.5 g) was separated on a RP-18 column, eluting with acetone/water (4:1) and futher purified on HPLC with aqueous acetonitrile (35%) to yield **6** (16.6 mg).

- Ilexkudinoside T (**1**): Molecular formula: $C_{47}H_{74}O_{17}$; White amorphous powder; HR-ESI-MS *m/z*: 945.4613 $[M+Cl]^-$ (calcd. for $C_{47}H_{74}O_{17}Cl$, 945.4615); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- Kudinoside D (**2**): Molecular formula: $C_{47}H_{72}O_{17}$; White amorphous powder; HR-ESI-MS *m/z*: 943.4478 $[M+Cl]^-$ (calcd. for $C_{47}H_{72}O_{17}Cl$, 943.4458); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- Randiasaponin IV (**3**): Molecular formula $C_{47}H_{76}O_{17}$: White amorphous powder; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1.

- Kudinoside G (**4**): Molecular formula $C_{53}H_{86}O_{22}$; White amorphous powder; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 2.

- Ilexkudinoside W (**5**): Molecular formula $C_{53}H_{86}O_{21}$; White amorphous powder; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 2.

- Lactifoloside G (**6**): Molecular formula $C_{59}H_{96}O_{26}$; White amorphous powder; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 2.

III. Results and Discussion

The rhizomes of *A. ascalonicum* was extracted with methanol and partitioned with dichloromethane. The water layer usually expected rich of saponins which was then chosen for isolation using combination of chromatographic methods to give six compounds **1-6** (Fig. 1).

Compound **1** was isolated as a white amorphous powder. HR-ESI-MS analysis of **1** revealed quasi-molecular ion peak at *m/z* 945.4613 $[M+Cl]^-$ indicating a molecular formular of $C_{47}H_{74}O_{17}$ (calcd. for $C_{47}H_{74}O_{17}Cl$, 945.4615). The ^{1}H-NMR and HSQC spectra of **1** showed signals corresponding to seven tertiary methyl groups [δ_H 1.40, 1.35, 1.19, 1.05, 0.97, 0.89, 0.86 (each, 3H, s)], a secondary methyl group [δ_H 1.24 (3H, d, J = 6.0 Hz)], three anomeric protons [δ_H 5.23 (1H, br s), 4.54 (1H, d, J = 5.0 Hz), and 4.52 (1H, d, J = 7.5 Hz)], and an olefinic proton [δ_H 6.02 (1H, br s)]. The ^{13}C-NMR and HSQC spectra of **1** observed signals of 47 carbons including nine non-protonated carbons, 19 methine groups, 11 methylene groups, and eight methyl groups. Above NMR data suggested that **1** to be a triterpene saponins. Futhermore, an ursane-type triterpene was recoginized by HMBC experiment (Fig. 2), including correlations between H_3-23 (δ_H 1.05)/ H_3-24 (δ_H 0.89) and C-3 (δ_C 89.6)/ C-4 (δ_C 40.4)/ C-5 (δ_C 57.4), H_3-25 (δ_H 0.97) and C-1 (δ_C 39.7)/ C-5 (δ_C 57.4)/ C-9 (δ_C 49.0)/ C-10 (δ_C 38.3), H_3-26 (δ_H 0.86) and C-7 (δ_C 34.2)/ C-8 (δ_C 41.0)/ C-9 (δ_C 49.0)/ C-14 (δ_C 43.0), H_3-27 (δ_H 1.19) and C-8 (δ_C 41.0)/ C-13 (δ_C 136.4)/ C-14 (δ_C 43.0)/ C-15 (δ_C 25.7), H_3-29 (δ_H 1.40) and C-18 (δ_C 50.7)/ C-19 (δ_C 73.8)/ C-20 (δ_C 86.9), and H_3-30 (δ_H 1.35) and C-19 (δ_C 73.8)/ C-20 (δ_C 86.9)/ C-21 (δ_C 31.7). A deshielded carbon signal (δ_C 181.3) was assigned for carbonyl carbon C-28. Two olefinic carbons (δ_C 126.9 and 136.4) and

TABLE I. ^{1}H- AND ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **1-3** IN CD_3OD

No.	**1**		**2**		**3**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	39.7	1.63 (m)/1.04 (m)	39.5	1.95 (m)/1.08 (m)	40.0	1.65 (m)/1.02 (m)
2	27.1	1.82 (m)/1.72 (m)	27.1	1.90 (m)/1.80 (m)	27.0	1.81 (m)/1.70 (m)
3	89.6	3.19 (dd, 11.5, 4.5)	89.5	3.22 (dd, 9.0, 4.0)	90.8	3.14 (dd, 12.0, 4.5)
4	40.4	-	40.5	-	40.3	-
5	57.4	0.85 (m)	56.5	0.85 (m)	57.1	0.80 (m)
6	19.5	1.62 (m)/1.47 (m)	19.2	1.65 (m)/1.48 (m)	19.5	1.56 (m)/1.41 (m)
7	34.2	1.45(m)/1.49 (m)	33.8	1.41 (m)	34.1	1.54 (m)/1.35 (m)
8	41.0	-	43.2	-	41.3	-
9	49.0	1.80 (m)	55.6	2.06 (br s)	49.0	1.70 (m)
10	38.3	-	37.6	-	37.9	-
11	25.0	2.16 (m)/1.85 (m)	127.7	7.03 (dd, 10.5, 2.0)	24.8	1.98 (m)
12	126.9	6.02 (br s)	129.5	5.84 (br d, 10.5)	129.7	5.35 (br t, 3.0)
13	136.4	-	142.7	-	139.6	-
14	43.0	-	43.2	-	42.6	-
15	25.7	1.72 (m)/1.17 (m)	26.4	1.60 (m)/1.16 (m)	29.7	1.85 (m)/1.02 (m)
16	26.5	2.40 (m)/1.44 (m)	26.8	2.30 (m)/1.40 (m)	26.5	2.20 (m)/1.85 (m)
17	41.6	-	44.6	-	49.0	-
18	50.7	2.24 (br s)	134.3	-	55.0	2.54 (s)
19	73.8	-	75.1	-	73.7	-
20	86.9	-	87.1	-	42.9	1.37 (m)
21	31.7	2.30 (m)/1.50 (m)	29.0	2.32 (m)/1.66 (m)	27.2	1.80 (m)/1.70 (m)
22	27.4	1.40 (m)/1.40 (m)	33.4	1.80 (m)/1.90 (m)	38.3	1.80 (m)/1.64 (m)
23	28.6	1.05 (s)	28.3	1.05 (s)	28.6	1.04 (s)
24	17.1	0.89 (s)	16.7	0.88 (s)	17.1	0.88 (s)
25	16.2	0.97 (s)	18.8	0.97 (s)	16.0	0.97 (s)
26	16.6	0.86 (s)	17.0	0.76 (s)	17.6	0.80 (s)
27	23.5	1.19 (s)	18.9	1.04 (s)	24.6	1.34 (s)
28	181.3	-	177.8	-	178.6	-
29	26.4	1.40 (s)	23.4	1.40 (s)	27.1	1.22 (s)
30	20.1	1.35 (s)	19.4	1.36 (s)	16.6	0.95 (d, 6.0)
	3-O-Ara		*3-O-Ara*		*3-O-Ara*	
1′	105.1	4.54 (d, 5.0)	105.2	4.52 (d, 5.0)	104.7	4.58 (d, 5.0)
2′	75.3	3.90 (m)	75.3	3.90 (dd, 8.5, 5.0)	76.8	3.79 (dd, 9.0, 5.0)
3′	81.8	3.88 (m)	82.2	3.88 (br d, 8.5)	73.0	3.76 (br d, 9.0)
4′	68.5	4.04 (br s)	68.5	4.05 (br s)	68.3	3.80 (br s)
5′	64.5	3.89*	64.6	3.90*	63.6	3.90*
		3.52 (dd, 11.5, 2.0)		3.53 (br d, 11.5)		3.52 (dd, 11.5, 2.0)
	2′-O-Rha		*2′-O-Rha*		*2′-O-Rha*	
1″	102.0	5.23 (br s)	102.0	5.23 (br s)	102.1	5.11 (d, 1.5)
2″	72.1	3.93 (dd, 3.5, 1.5)	72.1	3.92 (br d, 3.0)	72.2	3.90 (dd, 3.0, 1.5)
3″	72.1	3.72 (dd, 9.0, 3.0)	72.1	3.71 (dd, 9.0, 3.0)	72.2	3.70 (dd, 9.0, 3.0)
4″	73.8	3.40 (m)	73.8	3.40 (t, 9.0)	73.8	3.33 (t, 9.0)
5″	70.3	3.87 (m)	70.3	3.88 (m)	70.2	3.82 (m)
6″	18.0	1.24 (d, 6.0)	18.0	1.24 (d, 6.5)	18.0	1.24 (d, 6.0)
	3′-O-Glc		*3′-O-Glc*		*28-O-Glc*	
1‴	104.3	4.52 (d, 7.5)	104.3	4.51 (d, 4.5)	95.8	5.34 (dd, 7.5)
2‴	75.0	3.30 (dd, 9.0, 7.5)	75.1	3.32 (dd, 9.0, 7.5)	73.9	3.40 (dd, 8.5, 7.5)
3‴	78.0	3.30 (t, 9.0)	78.0	3.40 (t, 9.0)	78.6	3.35 (t, 8.5)
4‴	71.2	3.36 (m)	71.2	3.36 (t, 8.5)	71.2	3.36 (m)
5‴	78.0	3.40 (m)	78.1	3.30 (m)	78.3	3.42 (m)
6‴	62.4	3.85 (dd, 11.5, 2.0)	62.4	3.87 (dd, 12.0, 2.0)	62.5	3.81 (dd, 12.0, 2.0)
		3.69 (dd, 11.5, 5.5)		3.71 (dd, 12.0, 5.0)		3.70 (dd, 12.0, 5.0)

*) Overlapped signal

HMBC correlations between H-12 (δ_H 6.02) and C-18 (δ_C 50.7) indicated location of C=C double bonds at C-12/C-13. Carbon chemical shift values of C-19 (δ_C 73.8) and C-20 (δ_C 86.9) suggested for their bearing oxygen atoms. Moreover, the downfield shifted of C-20 compared to C-19 suggested for the lactone linkage between C-28 and C-20. Other signals of 17 carbons were assigned for the two hexose and one pentose units. The presence of a secondary methyl group in sugar moiety (δ_C 18.0 and δ_H 1.24) was proposed for a rhamnopyranosyl group. A pentose moiety in which proton H-4′ (δ_H 4.04) appeared as broad singlet signals demonstrated for an arabinopyranosyl group. The last hexose unit was assiged for a glucopyranosyl group by their carbon chemical shitf values (δ_C 104.3, 75.0, 78.0, 71.2, 78.0, and 62.4). Connection in trisaccharide sequence and the connection between sugar and aglycone moiety were then elucidated by HMBC spectra. The HMBC correlation between Ara H-1′ (δ_H 4.54) and C-3 (δ_C 89.6) indicated that sugar moiety linked to C-3 of the aglycone. The HMBC correlation between Rha H-1″ (δ_H 5.23) and Ara C-2′ (δ_C 75.3) confirmed rhanopyranosyl group linked to C-2′ of arabinopyranosyl unit. And the HMBC correlation between Glc H-1‴ (δ_H 4.52) and Ara C-3′ (δ_C 81.8) confirmed glucopyranosyl group linked to C-3′ of

TABLE II. ^{1}H- AND ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **4-6** IN CD_3OD

No.	**4**		**5**		**6**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	40.2	1.65 (m)/1.03 (m)	40.0	1.68 (m)/1.04 (m)	40.2	1.66 (m)/1.03 (m)
2	27.2	1.80 (m)/1.70 (m)	27.0	1.78 (m)/1.72 (m)	27.2	1.80 (m)/1.70 (m)
3	89.8	3.17 (dd, 9.0, 4.0)	90.8	3.14 (dd, 11.5, 4.5)	89.9	3.18 (dd, 9.0, 4.5)
4	40.3	-	40.3	-	40.4	-
5	57.2	0.80 (br d, 11.5)	57.1	0.82 (m)	57.2	0.80 (m)
6	19.5	1.55 (m)/1.41 (m)	19.5	1.57 (m)/1.42 (m)	19.4	1.56 (m)/1.43 (m)
7	34.2	1.55 (m)/1.35 (m)	34.3	1.56 (m)/1.43 (m)	34.3	1.55 (m)/1.42 (m)
8	41.3	-	40.1	-	41.3	-
9	48.0	1.70 (m)	49.0		48.5	1.69 (m)
10	37.9	-	37.9	-	37.8	-
11	24.7	1.98 (m)	24.7	2.0 (dd, 9.0, 3.5)	24.7	2.00 (dd, 9.0, 3.5)
12	129.7	5.32 (t, 3.0)	129.7	5.33 (br s)	129.8	5.32 (t, 3.5)
13	139.6	-	139.5	-	139.5	-
14	42.9	-	41.3	-	42.7	-
15	29.7	1.85 (m)/1.02 (m)	29.9	1.73 (m)/1.12 (m)	29.9	1.72 (m)/1.13 (m)
16	26.6	2.25 (m)/1.70 (m)	26.7	2.61 (m)/1.58 (m)	26.7	2.63 (m)/1.60 (m)
17	49.0	-	48.7	-	49.5	-
18	55.0	2.54 (s)	55.3	2.50 (s)	55.2	2.51 (s)
19	73.7	-	73.7	-	73.7	-
20	42.6	1.42 (m)	42.7	1.43 (m)	42.7	1.43 (m)
21	27.2	1.80 (m)/1.70 (m)	27.0	1.85 (m)/1.27 (m)	27.1	1.72 (m)/1.26 (m)
22	38.3	1.80 (m)/1.64 (m)	38.2	1.64 (m)	38.2	1.64 (m)
23	28.7	1.04 (s)	28.7	1.04 (s)	28.7	1.04 (s)
24	17.3	0.88 (s)	17.1	0.87 (s)	17.3	0.88 (s)
25	16.1	0.97 (s)	16.1	1.00 (s)	16.1	0.98 (s)
26	17.6	0.80 (s)	17.8	0.80 (s)	17.8	0.80 (s)
27	24.6	1.34 (s)	24.5	1.25 (s)	24.5	1.36 (s)
28	178.6	-	178.5	-	178.5	-
29	27.1	1.22 (s)	27.0	1.21 (s)	27.1	1.21 (s)
30	16.6	0.95 (d, 6.0)	16.6	0.94 (d, 6.5)	16.6	0.93 (d, 7.0)
	3-O-Ara		*3-O-Ara*		*3-O-Ara*	
1′	105.1	4.54 (d, 5.0)	104.7	4.59 (d, 4.5)	105.1	4.54 (d, 5.0)
2′	75.2	3.90 (dd, 9.0, 5.0)	76.9	3.80 (dd, 8.5, 4.5)	75.2	3.91 (dd, 9.0, 5.0)
3′	82.1	3.88 (br d, 9.0)	73.0	3.76 (br d, 8.5)	82.1	3.88 (br d, 9.0)
4′	68.5	4.04 (br s)	68.3	3.80 (br s)	68.4	4.04 (br s)
5′	64.5	3.90*	63.6	3.86*	64.5	3.89*
		3.52 (dd, 11.5, 2.0)		3.50 (dd, 11.5, 3.0)		3.53 (dd, 11.5, 2.0)
	2′-O-Rha		*2′-O-Rha I*		*2′-O-Rha I*	
1″	102.0	5.22 (br s)	102.0	5.11 (d, 1.0)	102.0	5.22 (d, 2.0)
2″	72.1	3.23 (br d, 3.0)	72.0	3.95 (dd, 3.0, 1.0)	72.1	3.95 (dd, 3.0, 1.5)
3″	72.1	3.71 (m)	72.2	3.71 (dd, 9.0, 3.0)	72.6	3.70 (dd, 9.0, 3.0)
4″	73.8	3.33 (m)	73.6	3.40 (t, 9.0)	73.8	3.41 (t, 9.0)
5″	70.3	3.87 (m)	70.3	3.80 (m)	70.2	3.88 (m)
6″	18.0	1.24 (d, 6.0)	18.0	1.25 (d, 6.5)	18.0	1.23 (d, 6.0)
	3′-O-Glc I		*28-O-Glc*		*3′-O-Glc I*	
1‴	104.3	4.51 (d, 7.5)	95.2	5.39 (d, 7.5)	104.3	4.51 (d, 7.5)
2‴	75.0	3.30 (dd, 9.0, 7.5)	76.7	3.62 (dd, 8.5, 7.5)	75.1	3.32 (m)
3‴	78.0	3.30 (m)	79.4	3.57 (t, 8.5)	78.0	3.32 (m)
4‴	71.2	3.35 (m)	71.5	3.43 (m)	71.2	3.32 (m)
5‴	78.0	3.40 (m)	78.3	3.34 (m)	78.0	3.40 (m)
6‴	62.4	3.85 (dd, 12.0, 2.0)	62.6	3.78 (dd, 11.5, 2.0)	62.5	3.78 (dd, 11.5, 2.0)
		3.70 (dd, 12.0, 5.0)		3.68 (dd, 11.5, 5.0)		3.67 (dd, 11.5, 5.0)
	28-O-Glc II		*2‴-O-Rha II*		*28-O-Glc II*	
1⁗	95.8	5.34 (dd, 7.5)	101.5	5.43 (d, 1.5)	95.2	5.40 (dd, 7.5)
2⁗	73.9	3.40 (dd, 9.0, 7.5)	72.2	3.90 (dd, 3.0, 1.5)	76.7	3.62 (dd, 8.5, 7.5)
3⁗	78.3	3.40 (m)	72.2	3.71 (t, 9.0)	79.4	3.58 (t, 8.5)
4⁗	71.2	3.35 (m)	73.9	3.42 (t, 9.0)	71.5	3.40 (m)
5⁗	78.6	3.40 (m)	70.3	3.80 (m)	78.3	3.34 (m)
6⁗	62.4	3.85 (dd, 12.0, 2.0)	18.2	1.28 (d, 6.0)	62.4	3.87 (dd, 12.0, 2.0)
		3.70 (dd, 12.0, 5.0)				3.69 (dd, 12.0, 5.0)
					2⁗-O-Rha II	
1⁗′					101.5	5.43 (d, 1.5)
2⁗′					71.9	3.93 (dd, 1.5, 3.0)
3⁗′					72.1	3.70 (dd, 9.0, 3.0)
4⁗′					73.7	3.41 (t, 9.0)
5⁗′					70.3	3.80 (m)
6⁗′					18.2	1.27 (d, 6.0)

*) Overlapped signals

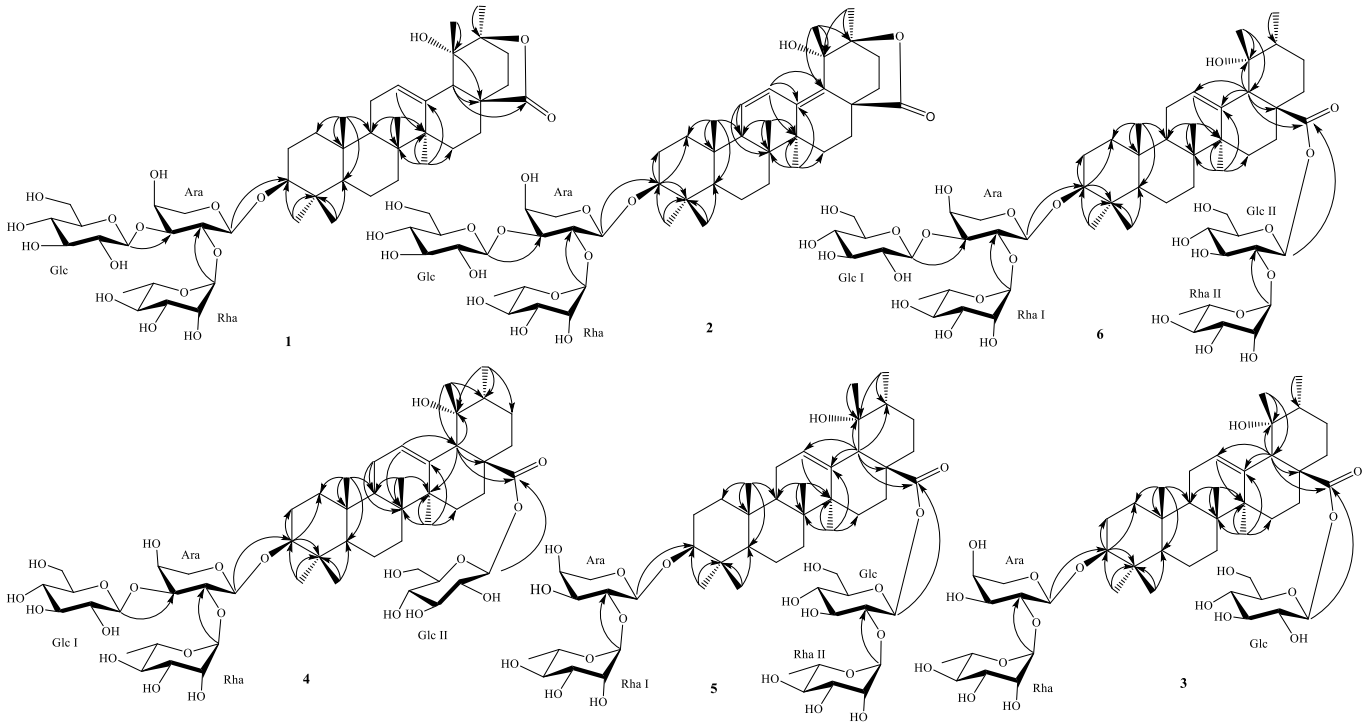

Fig. 2. *Important HMBC correlations (H→C) for compounds **1**-6*

arabinopyranosyl group. Consequently, the structure of **1** was established to be 3,19-dihidroxyurs-12-en-20,28-olide 3-*O*-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopy-ranosyl-(1→2)]-*α*-L-arabinopyranoside, a previous saponin isolated from the leaves of *Ilex kudingcha* and named as ilekudinoside T [9].

Compound **2** was isolated as a white amorphous powder. Molecular fomular of **2** was deduced to be $C_{47}H_{72}O_{17}$ by quassimolecular ion peak at *m/z* 943.4478 $[M+Cl]^-$ (calcd. for $C_{47}H_{72}O_{17}Cl$, 943.4458). The 1H and 13C-NMR spectral data of **2** were close similarity with those of **1** except the apperance signal of two olefinic protons [δ_H 7.03 (dd, 10.5, 2.0) and 5.84 (br d, 10.5)] and four olefinic carbons (δ_C 127.7, 129.5, 134.3, and 142.7). These evidences indicated that **2** contains two C=C double bonds insteat of one C=C double bonds in **1**. This deduction was also agreed by the loss of 2 hydrogen atoms from molecular formula of **2** ($C_{47}H_{72}O_{17}$) in comparison with that of **1** ($C_{47}H_{74}O_{17}$). The COSY correlation between two olefinic protons (δ_H 7.03 and 5.84) demonstrated for the presence of a vinylene group. Locations of these C=C double bonds were then clarified by HMBC experiment (Fig. 2). The HMBC correlations between H_3-29 (δ_H 1.40) and C-18 (δ_C 134.3)/ C-19 (δ_C 75.1)/ C-20 (δ_C 87.1) indicated the presence of C=C double bonds at C-13/C-18. And the HMBC correlations between H-12 (δ_H 5.84) and C-13 (δ_C 142.7)/ C-18 (δ_C 134.3), H-11 (δ_H 7.03) and C-13 (δ_C 142.7) demonstrated for an other C=C double bonds at C-11/C-12. Therefore, compound **2** was determined to be 3,19-dihidroxyursa-11,13(18)-diene-20,28-olide 3-*O*-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranoside. This compound was also previously isolated from *Ilex kudingcha* and named as kudinoside D [10].

The NMR spectral data of **3**-**6** (Table 2) indicated that they shared the same aglycone moiety and different in their sugar moiety. Different to **1** and **2**, a doublet proton signals of methyl group H_3-30 indicated that aglycone of **3**-**6** did not contain other substitutional group except methyl group (C-30). This deduction was confirmed by HMBC correlations between H_3-30 and C-19/ C-20/ C-22, and signals of methine group assigning for C-20 (Fig. 2). Therefore, aglycone of **3**-**6** was deduced to be 3,19-dihydroxyurs-12-en-28-oic acid. Not only showing HMBC correlation between Ara H-1′ and C-3, the HMBC spectra of **3**-**6** also observed addional HMBC correlations between another anomeric proton and carbonyl carbon C-28, indicating the presence of a glycosidic ester at C-28. In particulally, in the HMBC spectrum of **3**, correlation between Glc H-1‴ (δ_H 5.34) and C-28 (δ_C 178.6) indicated the glucopyranosyl group at C-28. Disaccharide moiety were assigned to be rhamnopyranosyl-(1→2)-arabinopyranosyl group, showing HMBC correlation bewteen Rha H-1″ (δ_H 5.11) and Ara C-2′ (δ_C 76.8). Connection of rhamnopyranosyl-(1→2)-arabinopyranosyl group at C-3 was confirmed by HMBC correlation between Ara H-1′ (δ_H 4.58) and C-3 (δ_C 90.8). Thus, compound **3** was determined to be 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl-19-hydroxyurs-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester, a saponin previously isolated from *Randia formosa* and named as randiasaponin IV [11]. The ^{1}H-NMR spectra of **4** and **5** showed an additional anomeric proton in comparison with **3**, indicating for the presence of four monosaccharides. Moreover, ^{13}C-NMR spectral data of **4** indicated the presence of a trisaccharide sequence, *β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl group as shown in compounds **1** and **2**, which was confirmed by HMBC

correlations between Glc I H-1‴ (δ_H 4.51) and Ara C-3′ (δ_C 82.1), Rha H-1″ (δ_H 5.22) and Ara C-2′ (δ_C 75.2). Connection of this trisaccharide group with aglycone at C-3 was demonstrated by HMBC correlation between Ara H-1′ (δ_H 4.54) and C-3 (δ_C 89.8). The second glucopyranosyl group at C-28 was confirmed by HMBC correlation between Glc II H-1⁗ (δ_H 5.34) and C-28 (δ_C 178.6). Thus, compound **4** was determined to be 3-*O*-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl -19-hydroxyurs-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester, a saponin previously isolated from *Ilex species such as I. kudingcha, I. latifolia* and named as kudinoside G [10, 12]. Sugar moieties of **5** were recognized by two individual disaccharide sequences. The HMBC correlation between Rha I H-1″ (δ_H 5.11) and Ara C-2′ (δ_C 76.9), Ara H-1′ (δ_H 4.59) and C-3 (δ_C 90.8) indicated the first disaccharide group, rhamnopyranosyl-(1→2)-arabinopyranosyl at C-3. The second disaccharide group was elucidated to be rhamnopyranosyl-(1→2)-arabinopyranosyl group and its linked to C-28 which were confirmed by HMBC correlations between Rha II H-1⁗ (δ_H 5.43) and Glc C-2‴ (δ_C 76.7), Glc H-1‴ (δ_H 5.39) and C-28 (δ_C 178.5). Thus, compound **5** was determined to be 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl-19-hydroxyurs-12-en-28-oic acid 28-*O*- *α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester, a saponin previously isolated from *Ilex kudingcha* and named as ilekudinoside W [13]. Compound **6** contained five monosaccharide units which characterized by five anomeric protons (δ_H 4.51, 4.54, 5.22, 5.40, and 5.43). Of these, presence of a trisaccharide sequence, *β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl group at C-3 was indicated by HMBC correlations between Glc I H-1‴ (δ_H 4.51) and Ara C-3′ (δ_C 82.1), Rha H-1″ (δ_H 5.22) and Ara C-2′ (δ_C 75.2), Ara H-1′ (δ_H 4.54) and C-3 (δ_C 89.9). Other disaccharide sequence, α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl group, at C-28 was confirmed by HMBC correlations between Rha II H-1⁗ (δ_H 5.43) and Glc C-2‴ (δ_C 76.7), Glc H-1‴ (δ_H 5.40) and C-28 (δ_C 178.5). Therefore, compound **6** was determined to be 3-*O*- *β*-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranosyl -19-hydroxyurs-12-en-28-oic acid 28-*O*- *α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester, a saponin previously isolated from *Ilex latifolia* and named as latifoloside G [14]

In summary, six ursane-type triterpene saponins including ilexkudinoside T (**1**), kudinoside D (**2**), randiasaponin IV (**3**), kudinoside G (**4**), ilexkudinoside W (**5**), lactifoloside G (**6**) were isolated from the rhizomes of *Allium ascalonicum*. Triterpene saponins have been reported potential cytotoxic and anti-inflamatory activities. Therefore, the identification of those saponins in *A. ascalonicum* may support for explanation of anti-inflammation and anti-tumor properties of this medicinal plant.

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