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Evaluation of novel antioxidant triterpenoid saponins from the halophyte *Salicornia herbacea*

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ABSTRACT

As a part of an ongoing search for novel antioxidants from the salt marsh plants, bioactivity-isolation and structure determination of constituents from *Salicornia herbacea* were performed. One new triterpenoid saponin (**4**), along with three known saponins (**1**–**3**), has been isolated from *n*-BuOH fraction of *S. herbacea*. On the basis of the spectroscopic methods, the structure of the new saponin **4** was elucidated as 3β-hydroxy-23-oxo-30-noroleana-12, 20(29)-diene-28-oic acid 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside. Scavenging effects of saponins **1**-**4** were examined on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical and peroxynitrite. Particularly, saponin **3** exerted significant antioxidant activity on both authentic peroxynitrite and peroxynitrite generated from morpholinosydnonimine (SIN-1).

In recent years, the role of reactive oxygen/nitrogen species (ROS/RNS) has attracted great attention due to their oxidation processes occurring in various biological systems.[1,2] The ROS/RNS comprehends free radicals and non-radicals. For example, there are superoxide ($O_2^{\cdot -}$), hydroxyl ($HO^{\cdot}$), hydroperoxyl ($HO_2^{\cdot}$), nitric oxide ($^{\cdot}NO$) and nitrogen dioxide ($^{\cdot}NO_2$) radical in free radicals, and hydrogen peroxide (H_2O_2) and peroxynitrite anion ($ONOO^-$) in non-radicals, respectively.[1,3] Because oxidation processes of ROS/RNS usually progress under severe control by several mechanisms in the body, imbalanced oxidative stresses result in several diseases such as cancer, Alzheimer's disease, neurodegeneration and cardiovascular disease.[1,4–6] Therefore, there is a growing interest in searching for natural antioxidants due to their lower toxicities than synthetic antioxidants.[7–10]

Salicornia herbacea L. (also known as glasswort) is annual succulent herb of chenopodiaceae family and one of the most salt tolerant plants. It is growing on salt marshes and muddy seashores along the western coast of Korea[11–14] and is widespread in Europe, Japan and Iran.[15–17] It contains large amounts of minerals, essential amino acid and essential fatty acid.[18–21] It has been used as not only a seasoned vegetable, salad and fermented food in coastal areas of Europe, Japan and Korea, but also as folk medicine for disorders such as constipation, obesity and diabetes.[22,12] Recently, the consumption of the plants has been extended into the functional food and medicinal plant due to its beneficial effects.[20,21] However, studies on its secondary metabolite have rarely been reported.[9,14,23–26]

In our continuing search for bioactive compounds from the salt marsh plants, we recently reported the isolation and peroxynitrite-scavenging activities of two flavonoid glycosides from *S. herbacea*.[14] In addition to these two compounds, an ^1H NMR spectral analysis of C_{18} vacuum flash chromatographic fractions for the *n*-BuOH layer of crude extracts revealed the presence of a structurally different group of metabolites. Large-scale extraction of organic materials followed by bioactivity-guided separation using combined chromatographic techniques yielded four saponin derivatives. Herein we report the isolation and structure determination of one new saponin (**4**), together with three known ones (**1**–**3**) (Fig. 1) and their DPPH radical and peroxynitrite scavenging activities.

The data were presented as mean ± SD. Differences between the means of the individual groups were assessed by one-way ANOVA with Duncan's multiple range tests. Differences were considered significant at $p < 0.05$.

The collected halophyte *S. herbacia* was processed to yield four solvent-partitioned fractions including *n*-hexane, 85% aqueous (aq)

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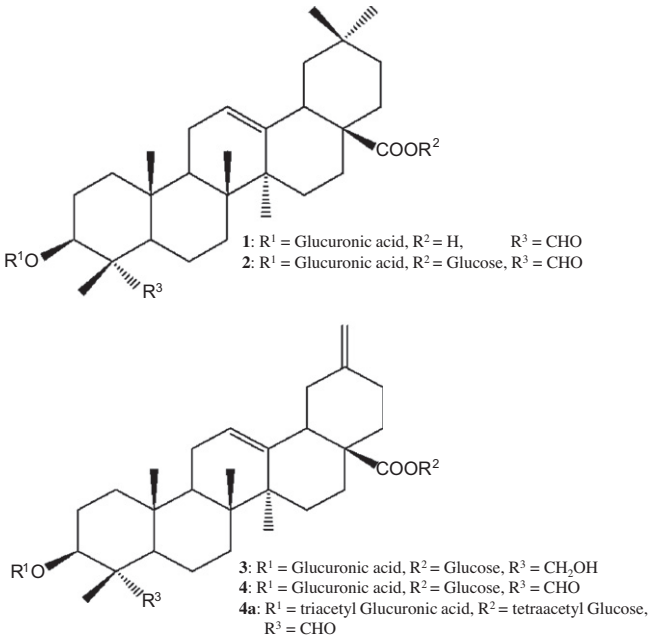

Figure 1. Chemical structure of saponins **1**–**4** isolated from *Salicornia herbacea*.

MeOH, *n*-BuOH and water.[27] Their antioxidant activities were assessed by measuring the DPPH radical and peroxynitrite scavenging activities.[28,29] Among the solvent fractions, 85% aq MeOH and *n*-BuOH fractions showed potent DPPH·scavenging activity with IC_{50} values of 15.6 and 40.9 μg/mL, respectively, which is comparable with positive control butylated hydroxytoluene (BHT) (IC_{50}, 39.8 μg/mL) (Table 1). Also, scavenging ratios of 85% aq MeOH and *n*-BuOH fractions were 70.8% and 71.6% for authentic peroxynitrite, and 99.5% and 99.8% for induced peroxynitrite from SIN-1 at a concentration of 50 μg/mL, respectively, as reported in our previous study (Fig. 2).[14] Induced peroxynitrite is $ONOO^-$ formed by ·NO and $O_2^{\cdot-}$, which are generated from SIN-1.

To find the possible scavenging components on ROS/RNS, further purification of *n*-BuOH fraction followed by bioactivity-guided separation using combined chromatographic techniques yielded four saponins (**1**–**4**),[30,31] including one new saponin. The structures of the isolated saponins were identified on the basis of spectroscopic analyses including ^1H and ^{13}C NMR spectroscopy, and verified by comparison with reported spectral data.[32] The chemical structures of the purified compounds are illustrated in Fig. 1.

Saponins **1**–**3** were identified by a combination of spectroscopic analysis and comparison with the literature data as gypsogenin 3-*O*-β-D-glucuronopyranoside, gypsogenin 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside, and 30-norhederagenin 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside, respectively.[32–37]

Table 1
DPPH radical scavenging activities of solvent fractions from *Salicornia herbacea*

	Concentrations (μg/mL)			IC_{50} (μg/mL)
	10	50	100	
L-Ascorbic acid	94.9 ± 0.25[a,*]	95.1 ± 0.24[a]	95.0 ± 0.25[a]	10<<
BHA	63.2 ± 1.84[b]	79.1 ± 1.05[b]	86.9 ± 0.66[b]	10<<
BHT	39.7 ± 3.01[d]	54.9 ± 2.25[c]	68.0 ± 1.60[c]	39.8
H_2O fr.	16.1 ± 4.20[f]	19.7 ± 4.01[d]	25.7 ± 3.72[d]	—
n-BuOH fr.	33.8 ± 3.31[e]	52.4 ± 2.38[c]	85.2 ± 0.74[b]	40.9
85% aq MeOH fr.	49.3 ± 2.53[c]	56.9 ± 2.16[c]	70.0 ± 1.50[c]	15.6
n-Hexane fr.	15.6 ± 4.22[f]	19.6 ± 4.02[d]	27.7 ± 3.62[d]	—

[*] DPPH radical scavenging activities (%).
[a–f] Means with the different letters in the same concentration are significantly different (p <0.05) by Duncan's multiple range test.

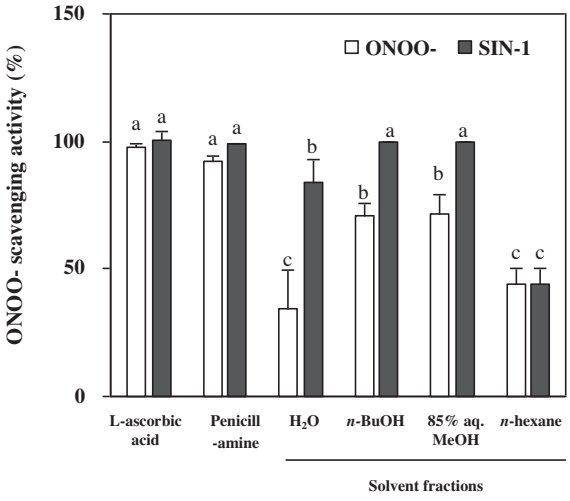

Figure 2. Peroxynitrite scavenging activities of each solvent fraction at a concentration of 50 μg/mL.[14] [a–c]Means with the different letters in the same reactive species are significantly different (p <0.05) by Duncan's multiple range test.

Saponin **4** was obtained as an amorphous white powder. Its molecular formula ($C_{41}H_{60}O_{15}$) was analyzed by a combination of negative HR-FABMS and ^{13}C NMR analyses. In the ^1H NMR spectrum, signals for two anomeric protons at δ 4.18 (1H, d, J = 7.7 Hz) and 5.36 (1H, d, J = 8.2 Hz) were correlated with signals for two carbons at δ 104.6 and 95.8 ppm by an HMQC experiment, and were diagnostic for the presence of two sugar molecules. Careful examination of the ^1H and ^{13}C NMR spectroscopic data revealed that saponin **4** possessed the same A–D rings as saponin **2**. However, there were significant differences in the ^{13}C NMR spectrum. Signals of two methyl carbons at δ 33.5 (C-29) and 24.0 (C-30), and quaternary carbon at δ 31.5 (C-20) in saponin **2** were replaced by the olefinic signals at δ 107.6 (CH_2) and 149.5 (C) in saponin **4**. Corresponding differences were found in the ^1H NMR spectrum, in which signals of methyl protons at δ 0.90 (s, H-29) and 0.92 (s, H-30) in saponin **2** were replaced by the exomethylene proton signals at δ 4.62 (1H, s) and 4.61 (1H, s) in saponin **4**. These differences were explained by formation of a double bond between C-20 and C-29, suggesting the aglycone part of saponin **4** as 3β-hydroxy-23-oxo-30-noroleana-12,20(29)-diene-28-oic acid, which has previously been reported.[38] This interpretation was also supported by comparison of the NMR spectral data with E ring moiety of saponin **3**. It was verified by sugar analysis and its NMR spectral data that the sugar moieties of saponin **4** were the same as those of **2** and **3**.

Acid hydrolysis of saponin **4** followed by TLC and direct comparison with standard sugars indicated the presence of glucose and glucuronic acid.[30] These results were confirmed by GC analysis of the alditol peracetates and by comparison to the standard. In addition, acetylation of saponin **4** resulted in a downfield shift of all sugar protons except those attached to carbons involved in the sugar-aglycone, which were then present in a much less overlapped part of the ^1H NMR spectrum. The cyclic structures of monosaccharides were determined to be D-glucuronopyranose and D-glucopyranose by 1D TOCSY and HMBC data on the acetylated product (saponin **4a**). A linkage of aglycone between D-glucuronopyranose and D-glucopyranose was assigned to be between C-3 and C-1′, and between C-28 and C-1″ on the basis of a long-range correlation in HMBC experiment, respectively. Thus, the structure of saponin **4** was unambiguously determined as 3β-hydroxy-23-oxo-30-noroleana-12,20(29)-diene-28-oic acid 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside.

Table 2
DPPH radical and peroxynitrite scavenging activities of saponins **1–4** isolated from *Salicornia herbacea*

	DPPH IC_{50} (mM)	Authentic $ONOO^-$ IC_{50} (μM)	SIN-1 IC_{50} (μM)
L-Ascorbic acid	<<0.02	1.5	<<1
BHA	<<0.1		
BHT	<<0.2		
Penicillamine		<<1	<<1
Saponin **1**	>>0.5	21.9	20.4
Saponin **2**	>>0.5	7.1	1.4
Saponin **3**	0.39	<<1	<<1
Saponin **4**	>>0.5	4.9	6.6

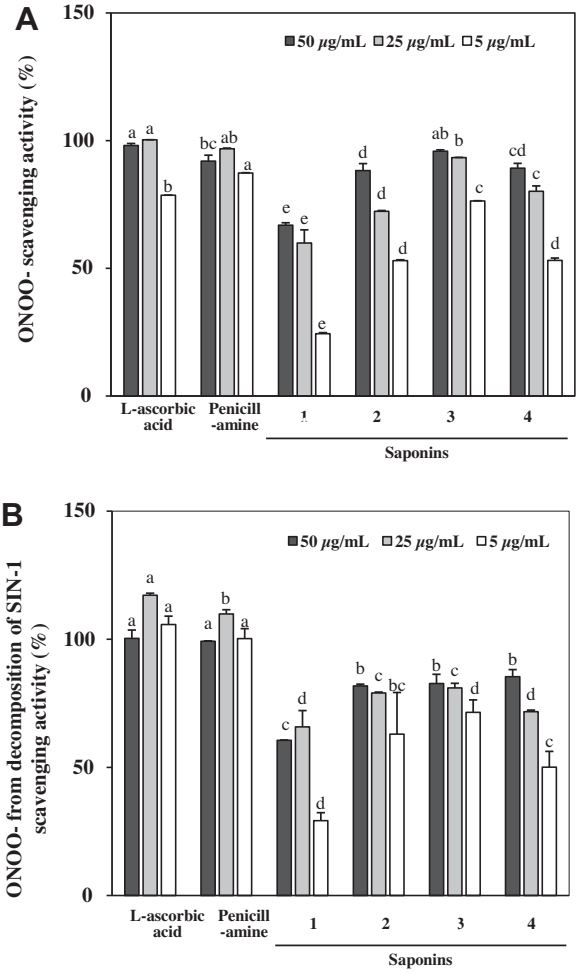

Figure 3. Peroxynitrite scavenging activities of saponins **1–4** on (A) authentic $ONOO^-$ and (B) induced $ONOO^-$ from SIN-1.[a–e]Means with the different letters in the same concentration are significantly different (p <0.05) by Duncan's multiple range test.

Potent antioxidant activity of four saponins isolated from the *n*-BuOH fraction was evaluated by measuring DPPH radical and peroxynitrite scavenging activities (Table 2). To the best of our knowledge, this is the first report on the DPPH· and $ONOO^-$ scavenging activities of these compounds. Saponins **1–4** showed relatively good activity on authentic $ONOO^-$ and induced $ONOO^-$ from morpholinosydnonimine (SIN-1), comparable with those of L-ascorbic acid and penicillamine, while they showed no significant effect on DPPH radical. Among saponins, activities of bidesmosidic saponins **2–4** on peroxynitrites were similar to each other at the concentration of 50 μg/mL, but that of monodesmosidic saponin (**1**) was relatively low (Fig. 3). Especially, saponin **3** exhibited the lowest value of IC_{50} (<<1 μM) for both authentic $ONOO^-$ and induced $ONOO^-$ from SIN-1. The activity differences between these derivatives were likely due to the number of hydroxy group present in each of them. Although it's difficult to explain clearly a scavenging effect of saponins on peroxynitrite, it's apparent that all of saponins **1–4** have much more scavenging effect on peroxynitrite than DPPH radical. Thus, the present study demonstrated that the peroxynitrite-scavenging effect of *S. herbacea* is at least partly attributed to saponins **1–4**. Furthermore, from 85% aq MeOH, purification of peroxynitrite-scavenging compounds is in progress.

In conclusion, it was found that *S. herbacea* is capable of scavenging the DPPH radical and peroxynitrite. In addition, four saponins (**1–4**), including one new saponin **4** were isolated from *n*-BuOH fraction by bioactivity-guided separation with chromatographic techniques. Their structures were determined by extensive 2D NMR experiments and by comparison with known compounds, and their antioxidant activities were evaluated on peroxyntrite. Saponin **3** showed the strongest scavenging effect on peroxynitrite. Therefore, this study suggests that saponins **1–4** have potential for their uses as natural antioxidants, which might be useful in the prevention of ROS/RNS and in the treatment of pathological processes such as aging, cancer and cardiovascular disease.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.017.

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27. *Extraction and isolation of bioactive compounds*: The Halophyte *Salicornia herbacea* was collected in Daebudo, Ansan, Korea in July 2004. The collected samples were briefly dried under shade and kept at −25 °C until use. The air-dried sample of *S. herbacea* (480 g) was chopped into small pieces and soaked for 2 days with MeOH (3 L × 2) and CH_2Cl_2 (3 L × 2), sequentially. The combined crude extracts (164 g) were evaporated under reduced pressure and partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aq MeOH and *n*-hexane, and then the aqueous layer was fractionated with *n*-BuOH and H_2O, successively, to afford the *n*-hexane (10.7 g), 85% aq MeOH (39.6 g), *n*-BuOH (53.7 g) and water (50.8 g) fractions. A portion of *n*-BuOH (50 g) fraction was separated into 6 subfractions by C_{18} (YMC–GEL ODS-A, 12 nm, S-75 μm) reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH and H_2O (50%, 60%, 70%, 80%, 90% aq MeOH and 100% MeOH). The fourth fraction was further separated on a silica gel column chromatography with gradient mixtures of MeOH and $CHCl_3$. The fraction eluted with 20% MeOH in $CHCl_3$ (1.078 g) was further separated by reversed-phase HPLC (YMC ODS-A, 80% aq MeOH, 1 cm x 25 cm, 2 mL/min) to yield saponin **1** (9.8 mg). The fraction eluted with 50% MeOH in $CHCl_3$ (0.548 g) was separated by reversed-phase HPLC (YMC ODS-A, 50% aq MeOH, 1 × 25 cm, 2 mL/min) to give saponins **3** (26.0 mg), **4** (13.5 mg), and **2** (9.8 mg) in order of elution.
28. *DPPH radical scavenging effect*: Aliquots of extracts or pure compounds in MeOH solution were added to DPPH· methanol solution (1.5×10^{-1} M). After gentle mixing and holding for 30 min at room temperature, the optical density was measured at 520 nm. L-ascorbic acid and penicillamine, BHA and BHT were used as a positive control.
29. *$ONOO^-$ scavenging ability by monitoring the $ONOO^-$-induced oxidation of dihydrorhodamine 123 (DHR 123) to rhodamine 123*: A stock solution of DHR 123 (5 mM) was purged with nitrogen and stored at −80 °C. A working solution of DHR 123 (final conc. 5 μM) diluted from the stock solution was placed on ice in the dark immediately prior to the determinations. The buffer containing 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4) and 5 mM potassium chloride was purged with nitrogen and placed on ice before use. At the beginning step of the experiments, diethylenetriaminepentaacetic acid (DTPA) (final conc. 100 μM) was added to the buffer. The $ONOO^-$ scavenging activity was determined at room temperature with a microplate fluorescence spectrophotometer FL 500 (Bio-Tek instruments, USA) using excitation and emission wavelengths of 485 and 530 nm. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (final conc. 10 μM) or authentic $ONOO^-$ (final conc. 10 μM) in 0.3 M sodium hydroxide. Oxidation of DHR 123 by decomposition of SIN-1 gradually increased whereas authentic $ONOO^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time. L-ascorbic acid and penicillamine were used as a positive control.
30. *Acid hydrolysis of saponins **2**–**4***: each compound (3 mg) in MeOH (2 mL) was individually treated with an equal volume of 1 N HCl under reflux for 2 h and then extracted with CH_2Cl_2 (3 × 20 mL). The aqueous was dried under a stream of nitrogen at 40 °C. Aliquots of 1 M NH_4OH and 5% $NaBH_4$ (each 1 mL) were added to each sample and the solution incubated at 40 °C for 90 min. After addition of AcOH (0.1 mL) to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and the AcOH codistilled with MeOH (2 × 10 mL). The resulting alditols were acetylated by refluxing for 6 h with Ac_2O/pyridine (1:1, 5 mL). The reaction mixture was quenched with water and extracted with CH_2Cl_2 and identified by GC-MS as those of glucose and glucuronic acid. Sapogenin of each compound was separated from each organic layer by preparative TLC on silica gel ($CHCl_3$–MeOH, 9:1) and confirmed by comparison with spectral data in the literature. *Gypsogenin 3-O-β-D-glucuronopyranoside* (**1**): white amorphous solid, mp 180–195 °C. $[\alpha]_D^{24}$ +16.7° (*c* = 0.72, MeOH). IR (KBr) υ_{max} cm^{-1}: 3340, 2938, 2810, 1690, 1470, 1300, 1215, 1020 and 680 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): 9.39 (1H, s, H-23), 5.24 (1H, t, *J* = 3.3 Hz, H-12), 4.24 (1H, d, *J* = 7.2 Hz, H-1′), 3.86 (1H, dd, *J* = 11.4, 4.3 Hz, H-3), 3.74 (1H, d, *J* = 9.1 Hz, H-5′), 3.46 (1H, dd, *J* = 9.6, 9.1 Hz, H-4′), 3.30 (1H, dd, *J* = 9.6, 9.1 Hz, H-3′), 3.11 (1H, dd, *J* = 9.1, 7.7 Hz, H-2′), 2.83 (1H, dd, *J* = 13.8, 3.9 Hz, H-18), 2.60–1.86 (4H, m, H-2, -11, -16), 1.84–1.63 (6H, m, H-1, -2, -9, -15, -19, -22), 1.63–1.42 (4H, m, H-6, -7, -16, -22), 1.42–1.26 (2H, m, H-5, -21,), 1.26–1.02 (5H, m, H-1, -7, -15, -19, -21), 1.18 (3H, s, H-27), 1.12 (3H, s, H-24), 1.00 (3H, s, H-25), 0.94 (3H, s, H-30), 0.91 (3H, s, H-29), 0.91 (1H, m, H-6), 0.82 (3H, s, H-26); ^{13}C NMR spectroscopic data[29]. Negative HR-FABMS *m/z*: 645.3637 $(M-H)^-$ (Calcd for $C_{36}H_{53}O_{10}$: 645.3639). *Gypsogenin 3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyranoside* (**2**): white amorphous solid, mp 213–230 °C. $[\alpha]_D^{24}$ −6.6° (*c* = 1.07, MeOH). IR (KBr) υ_{max} cm^{-1}: 3308, 2975, 2832, 1660, 1450, 1415, 1090, 1025, 880 and 630 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): 9.41 (1H, s, H-23), 5.36 (1H, d, *J* = 8.3 Hz, H-1″), 5.25 (1H, t, *J* = 3.2 Hz, H-12), 4.20 (1H, d, *J* = 7.8 Hz, H-1′), 3.92 (1H, dd, *J* = 11.5, 4.1 Hz, H-3), 3.81 (1H, br d, *J* = 11.2 Hz, H-6″), 3.67 (1H, dd, *J* = 11.2, 3.9 Hz, H-6″), 3.57 (1H, m, H-5″), 3.46–3.38 (3H, m, H-4′, -5′, -3″), 3.38–3.29 (3H, m, H-3′, -2″, -4″), 3.12 (1H, dd, *J* = 8.3, 7.8 Hz, H-2′), 2.84 (1H, dd, *J* = 13.2, 3.9 Hz, H-18), 2.08–1.85 (4H, m, H-2, -11, -16), 1.81–1.56 (7H, m, H-1, -2, -15, -16, -19, -22), 1.54–1.43 (2H, m, H-6, -7), 1.43–1.25 (2H, m, H-5, -21), 1.25–1.01 (4H, m, H-1, -7, -15, -21), 1.16 (3H, s, H-27), 1.09 (3H, s, H-24), 0.98 (3H, s, H-25), 0.92 (3H, s, H-30), 0.90 (3H, s, H-29), 0.83 (1H, m, H-6), 0.78 (3H, s, H-26); ^{13}C NMR spectroscopic data[29]. Negative HR-FABMS *m/z*: 807.4167 $(M-H)^-$ (Calcd for $C_{42}H_{63}O_{15}$: 807.4170). *30-Norhederagenin 3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyranoside* (**3**): white amorphous solid, mp 220–240 °C. $[\alpha]_D^{24}$ +3.7° (*c* = 1.14, MeOH). IR (KBr) υ_{max} cm^{-1}: 3336 (br), 2943, 2834, 1640, 1450, 1415, 1021 and 610 cm^{-1}. H-NMR (600 MHz, CD_3OD): 5.37 (1H, d, *J* = 8.2 Hz, H-1″), 5.32 (1H, t, *J* = 3.1 Hz, H-12), 4.62 (1H, s, H-29), 4.61 (1H, s, H-29), 4.44 (1H, d, *J* = 7.8 Hz, H-1′), 3.80 (1H, dd, *J* = 11.8, 1.5 Hz, H-6″), 3.68 (1H, m, H-3), 3.67 (1H, dd, *J* = 11.5, 4.8 Hz, H-6″), 3.64 (1H, d, *J* = 11.5 Hz, H-23), 3.46–3.29 (7H, m, H-3′, -4′, -5′, -2″, -3″, -4″, -5″), 3.27 (1H, d, *J* = 11.5 Hz, H-23), 3.26 (1H, dd, *J* = 9.2, 7.8 Hz, H-2′), 2.73 (1H, dd, *J* = 13.5, 4.1 Hz, H-18), 2.56 (1H, *J* = 14.5, 13.1, H-19), 2.26–2.11 (3H, m, H-16, -21), 2.08 (1H, dd, *J* = 13.5, 4.1, H-19), 1.98–1.87 (3H, m, H-2, -11, -22), 1.87–1.81 (2H, m, H-15, -16), 1.76 (1H, m, H-2), 1.66–1.46 (4H, m, H-6, -7, -9, -22), 1.40–1.21 (3H, m, H-5, -6, -7), 1.13 (1H, m, H-15), 0.98 (1H, m, H-6), 1.20 (3H, s, H-27), 0.98 (3H, s, H-25), 0.80 (3H, s, H-26), 0.70 (3H, s, H-24); ^{13}C NMR spectroscopic data[29]; HMBC correlations: H-3/C-4, C-23, C-24, C-1′; H-12/C-9, C-11, C-14, C-18; H-18/C-12, -13, -16, -17, -19, -28; H-19/C-18, -21, -20, -29; H-23/C-3, -4, -5, -24; H-24/C-3, -4, -5, -23; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-14, C-15; H-29/C-19, C-21; H-1′/C-3; H-2′/C-1′; H-1″/C-28; H-6″/C-4″, C-5″. Negative HR-FABMS *m/z*: 793.4013 $(M-H)^-$ (Calcd for $C_{41}H_{61}O_{15}$: 793.4010). *3β-Hydroxy-23-oxo-30-noroleana-12,20(29)-diene-28-oic acid 3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyranoside* (**4**): white amorphous solid, mp 235–245 °C. $[\alpha]_D^{24}$ +16.4° (*c* = 1.35, MeOH). IR (KBr) υ_{max} cm^{-1}: 3343 (br), 2930, 2850, 1670, 1560, 1210 and 670 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): 9.42 (1H, s, H-23), 5.36 (1H, d, *J* = 8.2 Hz, H-1″), 5.33 (1H, t, *J* = 3.3 Hz, H-12), 4.62 (1H, s, H-29), 4.61 (1H, s, H-29), 4.18 (1H, d, *J* = 7.7 Hz, H-1′), 3.95 (1H, dd, *J* = 11.6, 4.5 Hz, H-3), 3.80 (1H, br d, *J* = 11.5 Hz, H-6″), 3.67 (1H, dd, *J* = 11.5, 4.8 Hz, H-6″), 3.51 (1H, d, *J* = 9.8 Hz, H-5″), 3.43–3.37 (2H, m, H-3′, -3″), 3.36–3.29 (4H, m, H-3′, -2″, -4″, -5″), 3.12 (1H, dd, *J* = 9.2, 7.7 Hz, H-2′), 2.74 (1H, dd, *J* = 13.1, 4.6 Hz, H-18), 2.56 (1H, *J* = 13.5, 13.5 Hz, H-19), 2.26–2.11 (3, m, H-16, -21), 2.09 (1H, dd, *J* = 14.5, 4.6, H-19), 2.04 (1H, m, H-2), 1.96–1.88 (3H, m, H-11, -22), 1.86–1.73 (3H, m, H-2, -15, -16), 1.72–1.66 (2H, m, H-1, -9), 1.58–1.46 (3H, m, H-6, -7, -22), 1.35 (1H, br d, *J* = 10.2 Hz, H-5), 1.24 (1H, m, H-7), 1.12 (2H, m, H-1, -15), 0.91 (1H, m, H-6), 1.21 (3H, s, H-27), 1.10 (3H, s, H-24), 1.00 (3H, s, H-25), 0.81 (3H, s, H-26); ^{13}C NMR spectroscopic data[29]; HMBC correlations: H-3/C-2, C-4, C-23, C-24, C-1′; H-5/C-4, C-6, C-7, C-10, C-23, C-24; H-12/C-9, C-11, C-14, C-18; H-18/C-12, C-13, C-16, C-17, C-19, C-28; H-19/C-13, C-18, C-20, C-21, C-29; H-23/C-5, C-24; H-24/C-3, C-4, C-5, C-23; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-19, C-20, C-21; H-1′/C-3, C-3′, C-5′; H-2′/C-1′, C-3′; H-5′/C-3′, C-4′, C-6′; H-1″/C-28, C-3″; H-6″/C-4″, C-5″. Negative HR-FABMS *m/z*: 791.3819 $(M-H)^-$ (Calcd for $C_{41}H_{59}O_{15}$: 791.3854). *Peracetylation of saponin **4***: Saponin **4** (5 mg) in pyridine (0.3 mL) was treated separately with Ac_2O (0.2 mL) for 24 h at room temperature. After removing the pyridine and excess acetic anhydride under vacuum, the residue was purified by reversed-phase HPLC (100% MeOH) to yield **4a** (3.5 mg): a colorless gum; ^1H NMR (600 MHz, CD_3OD), δ 9.32 (1H, s, H-23), 5.68 (1H, d, *J* = 8.3 Hz, H-1″), 5.35 (1H, t, *J* = 3.0 Hz, H-12), 5.34 (1H, dd, *J* = 10.3, 8.3 Hz, H-3″), 5.17 (1H, dd, *J* = 9.8, 9.3 Hz, H-3′), 5.11 (1H, dd, *J* = 9.8, 9.3 Hz, H-4′), 5.06 (1H, dd, *J* = 8.3, 8.3 Hz, H-2″), 5.05 (1H, dd, *J* = 10.3, 10.3 Hz, H-4″), 4.81 (1H, dd, *J* = 9.3, 8.4 Hz, H-2′), 4.62 (1H, s, H-29), 4.61 (1H, d, *J* = 8.4 Hz, H-1′), 4.59 (1H, s, H-29), 4.27 (1H, dd, *J* = 12.5, 4.1 Hz, H-6″), 4.04 (1H, dd, *J* = 12.5, 2.2 Hz, H-6″), 3.98 (1H, dd, *J* = 10.3, 4.1, 2.2 Hz, H-5″), 3.89 (1H, d, *J* = 9.3 Hz, H-5″), 3.86 (1H, dd, *J* = 11.7, 4.4 Hz, H-3), 2.69 (1H, dd, *J* = 13.2, 4.4 Hz, H-18), 2.54 (1H, t, *J* = 13.2 Hz, H-19), 2.26–1.91 (6H, m, H-2, -11, -16, -19, -21), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 2.00 (3H, s, OAc), 1.99 (3H, s, OAc), 1.98 (3H, s, OAc), 1.97 (3H, s, OAc), 1.94 (3H, s, OAc), 1.86 (1H, m, H-22), 1.81–1.64 (4H, m, H-1, -2, -9, -16), 1.62–1.46 (3H, m, H-6, -7, -15), 1.43 (1H, m, H-22), 1.34 (1H, br d, *J* = 10.2 Hz, H-5), 1.21 (3H, s, H-27), 1.19 (3H, m, H-1, -7, -15), 0.97 (3H, s, H-25), 0.95 (3H, s, H-24), 0.88 (1H, m, H-6), 0.76 (3H, s, H-26).
31. ^{13}C NMR spectral assignments for saponins **1**–**4** from *Salicornia herbacea*.

No	**1**[a]	**2**[b]	**3**[a]	**4**[a]
1	39.2t	39.2t	39.6t	39.4t
2	25.5t	25.8t	26.3t	25.7t
3	83.0d	83.5d	82.2d	82.8d
4	56.3s	56.1s	43.9s	56.5s
5	48.9d	49.0d	48.2d	48.8d
6	21.4t	21.4t	24.2t	21.5t
7	33.2t	33.6t	33.4t	33.3t
8	41.0s	40.9s	40.7s	41.2s
9	48.9d	48.8d	49.0d	48.9d
10	36.9s	37.0s	37.7s	37.1s
11	23.9t	23.9t	24.6t	24.6t
12	123.5d	123.3d	124.3d	124.5d
13	144.8s	145.0s	144.3s	144.4s
14	43.0s	43.0s	43.0s	43.1s
15	28.8t	28.8t	28.9t	29.0t
16	24.5t	24.5t	24.2t	24.2t

(*continued on next page*)

(*continued*)

No	**1**[a]	**2**[b]	**3**[a]	**4**[a]
17	48.0s	47.6s	48.4s	48.3s
18	42.6d	42.7d	48.6d	48.6d
19	47.1t	47.2t	42.6t	42.7t
20	31.5s	31.6s	149.4s	149.5s
21	34.8t	34.9t	30.9t	31.0t
22	33.1t	33.3t	38.4t	38.5t
23	209.6d	208.7d	64.8t	209.5d
24	10.4q	10.5q	13.4q	10.5q
25	16.2q	16.1q	16.5q	16.3q
26	17.7q	17.7q	17.8q	17.9q
27	26.4q	26.5q	26.4q	26.5q
28	178.3s	181.5s	177.4s	177.5s
29	33.5q	33.8q	107.4t	107.6t
30	24.0q	24.0q	—	—
1′	104.5d	105.0q	105.0d	104.6d
2′	75.0d	74.8d	75.0d	75.3d
3′	77.6d	77.4d	78.1d	77.9d
4′	73.5d	73.0d	73.6d	73.7d
5′	76.6d	76.5d	76.5d	76.5d
6′	178.3s	172.5s	177.0s	177.0s
1″	95.7d	—	95.8d	95.8d
2″	73.8d	—	73.9d	74.0d
3″	78.1d	—	78.2d	78.3d
4″	71.0d	—	71.1d	71.2d
5″	78.6d	—	78.6d	78.8d
6″	62.3t	—	62.4t	62.6t

[a,b]Measured in CD_3OD at 150 and 75 MHz, respectively.

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