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ORIGINAL ARTICLE

New Bioactive C_{15} Acetogenins from the Red Alga *Laurencia obtusa*

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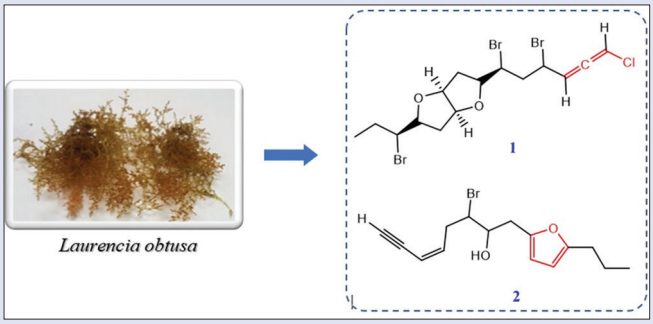
ABSTRACT

Background and Objective: With regard to the uniqueness of the red algae of the genus *Laurencia* as the source of C_{15}-acetogenins, along with the diversity of biological applications; the acetogenin content of the Red Sea *Laurencia obtusa* was investigated. **Materials and Methods:** Fractionation and purification of the CH_2Cl_2/MeOH extract were carried out by applying several chromatographic techniques, including column and preparative thin-layer chromatography; followed by a series of ^1H nuclear magnetic resonance measurements to give rise of some interesting notes. Toxicity to *Artemia salina* was evaluated. The apoptosis induced by these two compounds was demonstrated by DNA fragmentation assay and microscopic observation. **Results:** A new rare chloroallene-based C_{15} acetogenin, laurentusenin (1) along with a new furan ring containing C_{15} acetogenin, laurenfuresenin (2), were isolated from the red alga *L. obtusa*. Comparing 1D and 2D NMR, MS, ultraviolet and infrared radiation spectral data for the newly isolated compounds with the reported bromoallene containing acetogenins spectral data was played the crucial role for characterization of their chemical structures. 1 and 2 exhibited bare toxicity (LD50 >12 mM) in test organism, *A. salina* and induced apoptotic death confirmed by DNA fragmentation and microscopic investigations. **Conclusion:** The isolated metabolite 1 showed unusual substituted allene side chain, while 2 inserted furan ring as a new acetogenin nucleus. Both compounds may play a role in apoptosis induction and initiation and propagation of inflammatory responses.

Key words: Anti-inflammatory, fatty acids, haloethers, polyketides, red algae

SUMMARY

- A new rare chloroallene-based C_{15} acetogenin and a new furan ring containing C_{15} acetogenin were isolated from the red alga *Laurencia obtusa*.
- Both compounds may play a role in apoptosis induction and initiation and propagation of inflammatory responses.



Abbreviations used: NMR: Nuclear magnetic resonance; MS: Mass spectrometry; UV: Ultraviolet spectroscopy; IR: infrared radiation; EIMS: Electron ionization mass spectra; TLC: Thin-layer chromatography; PPP: Platelet poor plasma; PRPDS: platelet-rich plasma derived serum. DEPT: Destortionless Enhancement by Polarization Transfer; NOESY: Nuclear Overhauser Effect Spectroscopy; HSQC: Heteronuclear Single Quantum Coherence; HMBC: Heteronuclear Multiple-quantum Correlation; 1H-1H COSY: Correlation Spectroscopy.

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INTRODUCTION

Acetogenins are secondary metabolites originated from the polyketide pathway.[1] C_{15} acetogenins are entirely exclusive to members of the red algae of the genus *Laurencia* (*Rhodomelaceae*) and some of their herbivores as well.[2] The presence of one or more halogen atom (basically bromine atom), uncommon cyclic ethers with different ring sizes and a conjugated enyne or bromoallene terminal functions are the major features of the algal acetogenins.[3]

C_{15} acetogenins are generally categorized based on structural features, such as the presence of rings and their size, or the nature of the terminal group (conjugated enyne or bromoallene). The C_{15} acetogenins ring sizes varied from five-membered (tetrahydrofuran), six-membered (tetrahydropyran), seven-membered (oxepane or bear additional cyclopropane ring 6, 9-epoxide ring), eight-membered (ex. Laurencin, the first reported acetogenin), to 9–12-membered (epoxy ring is frequently encountered in this division) cyclic ethers.[3]

With regard to the acetogenin stunning structures, biological activities,[3-5] their role as chemotaxonomic markers,[6] and as a part of our ongoing search for more new structures and/or biological active factors from the Red Sea marine milieu;[7-11] the acetogenin content of the red alga *L. obtusa* collected near Jeddah's coast was investigated. Moreover, as a continuation of our previous studies,[9-11] the anti-inflammatory effect of the newly isolated compounds was estimated.

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MATERIALS AND METHODS

General

Column chromatography was performed with Aluminum Oxide Fluka, neutral type 507C. Fractions were examined by thin layer chromatography (TLC) F254 Si-gel plates. Preparative TLC glass plate (20 cm × 20 cm) supported silica gel of 250 μm thickness was used. Spots were visualized using ultraviolet (UV) light (254 nm) then detected by using spray reagent *p*-anisaldehyde-sulfuric acid. Sephadex LH-20 (GE Healthcare) with particle size 18–111 μm was utilized. Electron ionization mass spectra (EIMS) and High-resolution electron impact mass spectra were recorded on Krators EIMS-25 instrument at ionizing voltage of 70 eV. The 1D and 2D Nuclear Magnetic Resonance data were obtained on Bruker 850 MHz spectrometer. Samples were dissolved in deuterated chloroform $CDCl_3$ (δ_H 7.26 and δ_C 77.0).

Plant material

Laurencia obtusa was collected in May 2016 from Salman Gulf, north of Jeddah, KSA. Voucher sample (JAD 03060) was deposited at the Marine Chemistry Department, King Abdulaziz University, Jeddah, Saudi Arabia. The sample was identified by Prof. Mohsen El-sherbiny, Marine biology Department, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

Extraction and isolation

L. obtusa was air-dried (200 g) then extracted with equal volume of dichloromethane/methanol. The residue (6 g) was applied in column chromatography of aluminum oxide using gradient elution *n*-hexane/diethyl ether; then *n*-hexane/ethyl acetate. Fractions of 25 ml were gathered and monitored by using TLC technique and visualized using UV light (254 nm) then detected by using spray reagent *p*-anisaldehyde-sulfuric acid. The analogous and promising fractions were collected. The fraction eluted with *n*-hexane: diethyl ether (8.5:1.5) was purified by Sephadex LH-20 with MeOH: $CHCl_3$ (9.5:0.5) and then by preparative TLC system using *n*-hexane: diethyl ether (8.5:1.5). The yellow color zone with *p*-anisaldehyde-sulfuric acid was collected to provide 1 (1.5 mg). Another fraction eluted with *n*-hexane: ethyl acetate (6:4) was purified by Sephadex LH-20 with MeOH: $CHCl_3$ (9:1) and then by preparative TLC system using *n*-hexane: ethyl acetate (7:3). The green color zone with *p*-anisaldehyde-sulfuric acid was collected to provide 2 (2.7 mg).

Characterization of the isolated compounds

Laurentusenin (1); colorless oil (1.5 mg); R_f = 0.21; $[\alpha]_D$ = −27.60 (*c* 0.015, CH_2Cl_2); UV (MeOH) λ_{max} 201 (3.85) nm; infrared radiation (IR) ν_{max} 2922, 2852, 1961, 1737, 1463, 1378, 1272, 1166 cm^{-1}; ^1H NMR ($CDCl_3$, 850 MHz) and ^{13}C NMR $CDCl_3$, 212.5 MHz [Table 1]; ESI-HRMS *m/z* 526.8587, 530.8566, 530.8536 $[M + Na]^+$ (39.9:42.2:37.4) (calcd. For $C_{15}H_{20}{}^{79}Br_3{}^{35}ClO_2Na$, 526.8600; $C_{15}H_{20}{}^{79}Br_2{}^{81}Br{}^{35}ClO_2Na$, 528.8579; $C_{15}H_{20}{}^{79}Br_2{}^{81}Br{}^{37}ClO_2Na$ 530.8550).

Laurenfuresenin (2); pale yellow oil (2.7 mg); R_f = 0.27; $[\alpha]_D$ = −95.45 (*c* 0.022, CH_2Cl_2); UV (MeOH) λ_{max} 207 (4.5), 222 (42.52), 230 (5.05), 242 (4.83) nm; IR ν_{max} 3424, 3296, 2962, 2929, 2854, 1720, 1666, 1613, 1569, 1460, 1380, 1248, 1163 cm^{-1}; ^1H NMR ($CDCl_3$, 850 MHz) and ^{13}C NMR ($CDCl_3$, 212.5 MHz) [Table 1]; ESI-HRMS *m/z* 333.0453, 335.0433 $[M + Na]^+$ (100:98) (calcd. for $C_{15}H_{19}{}^{79}BrO_2Na$, 333.0466; $C_{15}H_{19}{}^{81}BrO_2Na$, 335.0446).

Biological evaluation of the isolated compounds

Preparation of blood neutrophils

Neutrophils (>98% pure on May-Giemsa stain) were isolated from peripheral blood of normal healthy volunteer donors by a combination of dextran sedimentation and centrifugation through discontinuous plasma Percoll gradients.[12] In brief, neutrophils were prepared as follows: Freshly drawn venous blood was citrated (1.1 ml of 3.8% sodium citrate to 10 ml blood), centrifuged at 300 g for 20 min at 20°C and the platelet-rich plasma aspirated and centrifuged at 2500 g for 10 min (for the production of platelet poor plasma [PPP]) or recalcified by adding 20 mM final concentration of calcium chloride to prepare platelet-rich plasma derived serum (PRPDS), to red and white cells remaining in each tube 5 ml of 6% dextran (500,000 mol wt) in 0.9% saline mixed gently and then allowed to stand for erythrocyte sedimentation for 30 min. The leukocyte-rich plasma was aspirated, centrifuged at 275 g for 6 min, suspend in 2 ml. The leukocytes were then mix with 2 ml of 42% percol (9:1 vol/vol percol-0.9% saline) in PPP followed by adding 2 ml of 51% percol in PPP. The gradients were centrifuged at 275 g for 10 min, and neutrophils were then aspirated from the interface of the 51% and 42% percol.

Table 1: ^1H and ^{13}CNMR spectral data for compounds 1 and 2

Position	1			2		
	δ_H	Multiplicity *J* in Hz	δ_C	δ_H	Mult. *J* in Hz	δ_C
1	6.26	dd, 6.0, 1.7	91.9	3.15	d, 1.7	82.9
2	-		201.0	-		79.8
3	5.88	dd, 6.8, 6.0	103.0	5.61	ddt, 11.1, 2.6,0.9	111.1
4	4.83	tdd, 10.2, 6.0, 1.7	56.5	6.09	dddd, 11.9, 7.7, 6.8, 0.9	141.2
5	2.36-2.39	m	42.7	3.00-3.06	m	36.4
	2.20	ddd, 12.8, 11.1, 1.7				
6	4.38	ddd, 11.1, 4.3, 1.7	53.9	4.10	ddd, 8.5, 6.0, 3.4	60.1
7	4.03	ddd, 12.8, 7.7, 6.8	83.1	3.84	brd, 4.3	71.8
8	2.06	ddd, *J*=9.4, 7.7, 1.7	37.3	2.90-2.96	m	34.9
	2.28-2.33	m				
9	4.63	ddd, 8.5, 4.3, 2.6	85.1	-		149.2
10	4.49	ddd, 6.0, 4.3, 1.7	84.5	6.04	d, 2.6	108.0
11	2.33-2.37	m	37.5	5.89	d, 2.6	105.5
12	4.13	m	83.7	-	-	155.8
13	4.17	td, 8.5, 2.6	61.4	2.55	t, 7.7	30.1
14	2.11-2.15	Ha, m	28.2	1.62-1.66	m	21.4
	1.73-1.76	Hb, m				
15	1.06	t, 6.8	11.5	0.94	t, 7.7	13.8

Culture of neutrophils

Neutrophils were prepared as described above, then resuspended in an appropriate volume of RPMI 1640 medium with 10% autologous PRPDS and 100 µ/L of penicillin and streptomycin and divided into five equal volumes each put in the culture tube. Cells were incubated (at 37°C in a 5% carbon dioxide) as follows:

1. Only cells
2. Cells + DMSO at 0.01% v/v
3. Cells + each compound in DMSO at dose of 50 mM/ml culture.

The age of neutrophils in culture was calculated at the start of culture at time zero (or baseline), 24 h, 48 h, and 72 h.

Assessment of cell viability

At time 0 and then at subsequent times, cells were removed from culture and counted on a hemocytometer. Cell viability was determined by trypan blue dye exclusion test; one volume of trypan blue (0.4% GiBCo) was added to 5 volumes of cells at room temperature for 5 min. The IC_{50} of isolated compounds were determined in comparison to dexamethasone.

Measurements of apoptosis

The neutrophils apoptosis in each culture was assessed:

Morphological assessment of apoptosis

At time 0 and at subsequent times, cells were removed from each culture, fixed in methanol, harvested on slides and slides were stained with May-Grunwald-Giemsa and examined by oil immersion light microscope. For assessment of the percentage of cells showing morphology of apoptosis 500 cells/slide were examined for each case at different times (Zero, 24, 48, and 72 h) in the presence or absence of the drugs used. Neutrophils were considered apoptotic if they exhibited the highly characteristic morphological features of chromatin aggregation, nuclear pyknosis, and cytoplasmic vacuolation. The apoptotic neutrophils percentage at different times was calculated for normal cells in the presence or after addition of isolated compounds, and the results were then compared statistically using *F*-test and Student's *t*-test.

One drop from cell suspension was added to one drop of AO solution (10 µg/ml in phosphate buffered saline), mixed gently on a slide, and immediately examined with an Olympus HB-2 microscope with fluorescence attachment. Green fluorescence was detected between 500 and 525 nm. Cells exhibiting bright green fluorescent condensed nuclei (intact or fragmented) were interpreted as apoptotic cells and expressed as a percentage of the total cell number viable cells, were interpreted as cells which exhibited a green, diffusely stained intact nucleus.

DNA fragmentation assay

The assessment of chromatin fragmentation in neutrophils was done by modification of methods previously used for thymocytes. Cells (2.5 × 10.7) were washed three times and resuspended in a 0.15 mol/L NaCl solution. The cells were chilled at 4°C and lysed by adding 4.5 ml of 10 mmol/L of tris/HCl buffer, pH 8.0, containing 100 mmol/L of ethylenediaminetetraacetic acid (EDTA) and 0.2% volume/volume Triton X-100 (Lysis buffer). After 4 h, the lysate was centrifuged at 35,000 g at 4°C for 20 min. The supernatants were collected into tubes and precipitated with 0.1 volume of 5 mol/L of NaCl and 2 volumes of absolute ethanol. The DNA was precipitated for 24 h at 4°C. The precipitate was centrifuged at 12,500 g at 6°C for 15 min. The pellet was resuspended in 1 ml of 10 mmol/L of Tris HCl buffer pH 8 containing 100 mmol/L EDTA and 0.1 m ml/L of sodium dodecyl sulfate. Proteinase K was added to a final concentration of 20 mg/ml, and the sample was incubated for a further 24 h at 37°C. The DNA was extracted with phenol and chloroform and re-precipitated with absolute ethanol. The pellet was re-dissolved in 20 ml of lysis buffer and 10 µl of RNase. 1 and 2 were treated with neutrophils at 10, 20, or 40 mg/ml for 24 h. DNA was isolated as described in the text, electrophoresed on a 1% agarose gel, and stained with 0.5 mg/ml ethidium bromide, control (0.3% DMSO), 40 mg/ml of 1, 20 mg/ml of 2. Each sample of the purified DNA (20 µl) was subjected to electrophoresis in 1% agarose gel containing 200 ng/ml ethidium bromide and were visualized under UV light. The sizes of the fragments were confirmed by reference to a 1-Kb DNA ladder (Gibco/BRL).[12,13]

Toxicity of the isolated compounds

Toxicity of the 1 and 2 was detected using *Artemia salina* as test organism and DMSO as a negative control.[14] The toxicity of the tested material in DMSO, at varying concentrations were determined using brine shrimp larvae in seawater as the test organism. Brine shrimp larvae incubated in a mixture of seawater and DMSO were used as control. After 24 h of incubation at room temperature, the average number of larvae that survived in each vial was determined. The mean % mortality was plotted against the logarithm of concentrations, the concentration killing 50% of the larvae (LC_{50}) was determined from the graph.[14]

RESULTS AND DISCUSSION

The routine work of collection, extraction, and fractionation by applying several chromatographic techniques was accomplished and then followed by series ^{1}H NMR measurements for testing the compounds purity and also as a swift look to their class of natural metabolites. Among the aforementioned examined isolates, some of them were further subjected to several NMR analyses.

Among compounds of the genus *Laurencia*, it is customary to recognize the presence of the characteristic bromoallene terminus-containing acetogenins by observing a number of specific signals ^{13}C NMR spectrum (–HC^3=C^2=C^1HBr): δ_C of C-1 approximately 70.0 ppm, for C-2 almost 200.0, and about 100.0 ppm for C-3, along with the value of coupling constant (*J*) of the atoms H-1 and H-3.[3] A more confirmation came from the IR spectrum where the allene function (=C=C) absorbs at about 2000 cm^{-1}. However, the situation with the first isolated metabolite (1) was somewhat different. Besides bromoallenes, enyne-containing C_{15} acetogenins are most commonly isolated among *Laurencia* members; 2 showed a doublet signal at δ_H 3.15 (*J* = 1.7 Hz) and two signals belong to olefinic protons at 5.61 (ddt, *J* = 11.1, 2.6, 0.9) and 6.09 (dddd, 11.9, 7.7, 6.8, 0.9). Those signals indicated the presence of acetogenin which is normal, but, two doublet (*J* = 2.6 Hz) signals resonating at 6.04 and 5.89 ppm tempted us for more investigation of 2 which is then the first reported furan-based acetogenin [Figure 1].

Compound 1, was isolated as optically active colorless oil; its molecular formula was assigned as, $C_{15}H_{20}Br_3ClO_2$, from its HRESIMS (requires four unsaturations). The UV spectrum exhibited the presence of allene group absorbs at 201 nm. ^{1}HNMR spectrum revealed the presence of certain features that indicate the nature of compound 1: a tertiary Methyl resonating at δ_H 1.06 (t, *J* = 6.8 Hz); nine protons resonating

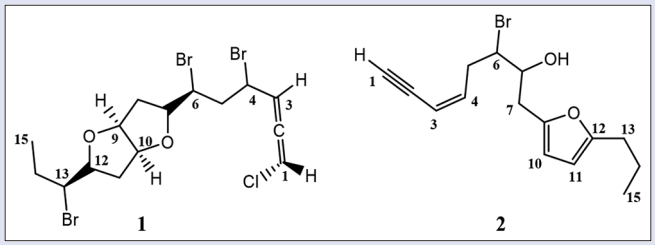

Figure 1: Acetogenins from *Laurencia obtuse*

in the range of δ_H 4.03–6.26 ppm; together with the absence of methyl equivalent protons assigned the fatty acid-derived nature of compound 1. All carbons and protons were associated with the aid of HSQC NMR experiment. ^{1}H, ^{13}C, and DEPT NMR spectra [Table 1] presented signals accounted for the presence of one quaternary carbon resonating at δ_C 201.0; seven methines attached to either an oxygen or halogen atoms; two methines resonating at δ_H/δ_C 6.26 (dd, J = 6.0, 1.7 Hz)/91.9 and at 5.88 (dd, J = 6.8, 6.0 Hz)/103.0; and one tertiary methyl function. The formerly mentioned two down-field methine protons, together with the quaternary could be assigned as bromoallene terminus, especially after examining the IR spectrum which clarify the presence of =C=C function resonating at 1961 cm^{-1}.[3] However, the reported value for C-1 (≈δ_C 70.0) is extremely up-field than that found for 1 δ_C 91.9, with the absence of absorption due to hydroxyl function in the IR spectrum, suggesting replacing of the Br atom by a Cl one. The ^{1}H-^{1}H COSY NMR spectrum revealed the presence of one large spin sequence system. For simplicity, it was divided into three substructures: (a) from H-1 to H-6; where H-3, part of allene system is resonating at δ_H 5.88 is correlated with H-4 resonating at 4.83, which in turn is correlated with the methylene protons H_2-5 at 2.36–2.39 and at 2.20, as well as these protons are correlated with H-6 resonating at 4.38; (b) from H-15 to H-13, where the methyl protons H_3-15 signal resonating at δ_H 1.06 (t, J = 6.8 Hz) is correlated with the methylene protons H_2-14 at 2.11–2.15 and 1.73–1.76, as well as these protons are correlated with H-13 resonating at 4.17; and (c) the third substructure can be represented as CHX(7)-CH_2(8)-CHX(9)-CHX(10)-CH_2(11)-CHX(12). The molecular formula showed the presence of two oxygen atoms which appeared as a part of ether linkages, this deduction was supported from the IR absorption at 1165 cm^{-1} and by examining the corresponding δ_C values. Hence, the third substructure contains a C-9-C-10 bond of fusion. Finally, the COSY strong correlation between H-6 and H-7, and that between H-12 and H-13, together with the correlations observed in the HMBC spectrum from H-8 and H-11 to both C-9 and C-10 supported the proposed substructure. The location and type of the electron withdrawing atoms were selected as Br atoms based on the values of δ_C of the three corresponding carbon signals at 56.5, 53.9 and 61.4 for C-4, C-6, and C-13, respectively, suggested the gross structure 1 [Figure 2]. The trivial name laurentusenin was given to compound 1. The relative configuration of 1 was determined by a combination of data from NOESY spectrum and the coupling constant values (*J*). NOESY enhancements were observed from H-9 (δ_H 4.63), H-10 (δ_H 4.49) to H-7 (δ_H 4.03) and H-12 (δ_H 4.13) suggesting the *cis*-orientation for H-12, H-10, H-9, and H-7. The relative configurations at C-9 and C-10 were assigned as identical to those of laurendificin[15] based on the chemical shift and coupling constant values, the biogenetic pathway that both compounds were isolated from the same algal genus as well as the optical rotation. Therefore, both H-9 and H-10 occupy α-orientation and the relative stereochemistry of the chloroallene function was assigned as *S** based on the method developed by Nader[16] while the relative configuration of C-4, C-6, and C-13 were unknown.

Compound 2, was isolated as pale yellow oil. HRESIMS established the molecular formula $C_{15}H_{19}BrO_2$, which requires 6 degrees of unsaturation. EIMS showed a characteristic molecular-ion cluster at *m/z* in 1:1 ratio, which clearly indicated the presence of one Br atom. The presence of acetylenic, hydroxyl, and furan ring were evidenced from the IR absorption at υ_{max} 3295, 3423, 1163, and 1070 cm^{-1}, respectively. ^{13}C NMR spectrum displayed 15 signals [Table 1], categorized by DEPT experiment into one methyl, four methylene, seven methine, and three quaternary carbons. ^{1}H, ^{13}C and HSQC NMR spectra assigned the following features: an acetylenic proton δ_H/δ_C 3.15/82.9; 4 olefinic methine protons 5.61/111.1, 5.89/105.5, 6.04/108.0, and 6.09/141.2; one hydroxylated methine 3.84 (brd, J = 4.3 Hz)/71.8; one halogenated methine 4.10 (ddd, J = 8.5, 6.0, 3.4 Hz)/60.1; four methylenes assigned to carbons resonating at δ_C 36.4, 34.9, 30.1, and 21.4; and a tertiary methyl at δ_H/δ_C 0.94 (J = 7.7 Hz)/13.8 carbons. Interpretation of the ^{1}H–^{1}H correlation spectroscopy spectrum showed the presence of three proton sequences: (a) the first one confirmed the presence of conjugated enyne group, in which, the olefinic (C-3) proton at δ_H 5.61 correlated to acetylenic (C-1) proton resonating at δ_H 3.15 and to the olefinic (C-4) proton at δ_H 6.09. H-4 is further correlated to the methylene (C-5) protons resonated at 3.00–3.06, as well as these are correlated with the (C-6) proton at δ_H 4.10. H-6 is correlated to the (C-7) proton resonated at 3.84 which in turn is correlated to the methylene (C-8) protons resonated at 2.90–3.96; (b) two olefinic methine proton signals resonating at 6.04 and 5.89 with coupling constant of 2.6 Hz; and (c) a sequence represents a propyl group residue where, a methyl protons resonating at δ_H 0.94 is correlated with the methylene protons resonated at 1.62–1.66 as well as this is correlated with the another methylene protons at δ_H 2.55.

From the previous discussion, 2 could be viewed as three substructures: 6-bromo-7-hydroxy octenyn-yl residue; propyl residue; and a 1,4-disubstituted furan (to fulfill six unsaturations and due to *J*-value between H-10 and H-11). Furthermore, the HMBC spectrum provides the way of attachments between the three moieties, where the methylene (C-8) protons signal is correlated with the carbon atoms signals at δ_C 149.2, 108.0, and 71.8, which establish the connection C-1 to C-10. The correlation between the methylene (C-13) protons signal is correlated with the carbon atoms signals resonating at δ_C 155.8, 105.5, and 21.4 [Figure 2].

The relative configuration of 2 was determined by the combination of data from NOESY spectrum and the coupling constant values (*J*). The *J*-value (11.1 Hz) between H-3 and H-4 indicated the *cis*-geometry of the double bond, which also showed a cross peak in NOESY spectrum, while the relative configuration of C-6 and C-7 were unknown. The trivial name laurenfuresenin was given to compound 2 [Figure 1].

In the current study, the IC_{50} of compounds 1 and 2 were determined by 24-h dose-response curve using peripheral blood neutrophils. Next, the recorded IC_{50} values were implemented to assess the time course of apoptosis, as shown in Table 2. These findings were further supported by assessing potential activities of the compounds on apoptotic cell death. It is generally accepted that the hallmark of apoptosis is oligo-nucleosomal degradation of DNA into 200 base pairs forming a pattern of the ladder on electrophoresis. On the other hand, in necrosis DNA is randomly

Table 2: Effect of the isolated compounds on apoptosis of peripheral blood neutrophils

Compound	Percentage apoptotic neutropils (mean±SD)			
	0 h	24 h	48 h	72 h
Control	0.41±0.01	0.41±0.01	0.41±0.01	0.41±0.01
1	0.50±0.09	18.10±1.43	23.07±1.97	25.89±2.74
2	0.95±0.61	31.01±1.88	48.62±2.54	75.13±2.21

Mean±SD (n=3, P<0.05). SD: Standard deviation

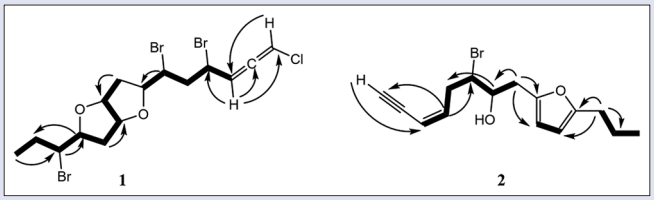

Figure 2: Selected COSY (▬) and HMBC (⌒) correlations of 1 and 2

fragmented forming smear of DNA materials on electrophoresis. To this end, neutrophils were cultured and treated with the isolated compounds [Table 2] for 24, 48 and 72 h. Then, the morphological and biochemical assays were undertaken to assess the percentage of apoptotic cell death in all cultures. The findings indicate that compound 2 exhibited the most potent apoptogenic potential. However, within the context of our experimental conditions, these findings do not rule out an involvement other modes of cell death such as necrosis, autophagy, and others.[17]

CONCLUSION

Two important metabolites have been isolated from the Saudi Red Sea red alga *L. obtusa*. One with unusual substituted allene side chain and the other inserts furan as a new class of acetogenins, namely laurentusenin (1) and Laurenfuresenin (2), respectively. These two metabolites displayed chemotaxonomic value to the genus *Laurencia*. The apoptosis induced by these two compounds was demonstrated by DNA fragmentation assay and microscopic observation. These observations suggest that 1 and 2 may be involved in the regulation of programmed death in the initiation and propagation of inflammatory responses.

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Conflicts of interest

There are no conflicts of interest.

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