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Original Research Article

In vitro Cytotoxic, Antibacterial and Antiviral Activities of Triterpenes from the Red Sea Sponge, *Siphonochalina siphonella*

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Abstract

Purpose: *To study the phytochemical composition of Siphonochalina siphonella sponge from the western coast of the Red Sea and to evaluate the isolates for possible in vitro cytotoxic, antibacterial and antiviral activities.*

Methods: *The compounds obtained were isolated and purified by different chromatographic means. Their structures were established by means of spectral analysis including 1D ^{1}H and ^{13}C and 2D correlation nuclear magnetic resonance (NMR) and high resolution mass spectroscopy (HR-MS). Crystal violet staining method (CVS) was used for the assessment of the cytotoxic activity against HepG-2 and MCF-7 human cell lines, while agar-well diffusion method was employed to measure antimicrobial activity against two Gram-positive and two Gram-negative bacteria. The antiviral activity was determined by the inhibition of cytopathic effect (CPE) in susceptible mammalian cells.*

Results: *Four triterpenes, possessing two different skeletons, were isolated and identified as sipholenone A, sipholenol A, neviotine A and sipholenol L. All four compounds were significantly cytotoxic to MCF-7 and HepG-2 cancer cell lines ($p < 0.05$) in a concentration-dependent manner with IC_{50} (the inhibitory concentration required to reduce cell survival by 50 %) in range 2.8 - 19.2 µg/mL. The highest antibacterial activity was observed for neviotine A (Compound 3) against Bacillis subtilis (17.2 ± 0.58). On the other hand, all the compounds showed moderate to weak anti-viral activity.*

Conclusion: *The findings reveal the strong cytotoxic activity of all the isolated triterpenes from the Red Sea Sponge, S. siphonella. Sipholenone A (Compound 1) displayed significant cytotoxic activity towards MCF-7 and HepG-2 cancer cell lines with IC_{50} = 3.0 and 2.8 µM, respectively.*

Keywords: *Red Sea Sponge, Siphonochalina siphonella, Triterpenes, Cancer cells, Cytotoxic, Antibacterial, Antiviral*

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INTRODUCTION

Sea organisms are a rich source of unique and diverse natural products with potent pharmacological activities, some of which are currently in preclinical or clinical trials [1]. Sponges (*Porifera*) are a predominantly marine phylum living in the intertidal and the abyssal (deepest ocean) zones. Worldwide, there are approximately 8,500 described species of sponges, with around eleven described from genus *Siphonochalina* [2]. Species of the Red

Sea sponge belonging to genus *Siphonochalina* are distributed throughout the Gulfs of Aqaba and Suez [3]. A potent hemiasterlin class of antitumor agents has been isolated from *Siphonochalina* spp. while an antifungal and antibacterial polyacetylene diol, siphonodiol, was reported from the marine sponge *Siphonochalina* truncate [4]. On the other hand, acetylated bile acid derivatives were obtained from the organic extracts of the sponge *S. fortis*, collected from Argentina shores [5]. *Siphonochalina siphonella*, a grey colonial tube-like sponge, is one of the few sponges known to produce squalene-derived cyclic ether triterpenes [3]. So far, about thirty compounds belonging to four different skeletal, namely, the sipholanes [6], siphonellanes [7], neviotanes [8] and dahabanes [3] have been reported. Several studies have described remarkable anti-proliferative activity of sipholane triterpenoids and some of their semisynthetic derivatives against a number of highly malignant cell lines [9]. Sipholenol A was found to potently reverse P-gp-mediated MDR (permeability glycoprotein-mediated multiple drug resistance) to colchicine, paclitaxel, and vinblastine in the resistant KB-C2 and KB-V1 cells overexpressing P-gp [10]. Biocatalysis of sipholenol A and sipholenone A were useful in getting new similar triterpenes with potent antiproliferative activity [11]. To the best of our knowledge, this is the first report assessing the antiviral activity; as well as the cytotoxic activity against HepG-2 cancer cell line, of *S. siphonella* triterpenoids from the Red Sea shores. Moreover, we report here the complete assignments of ^{1}H and ^{13}C NMR data of the isolated triterpenes.

EXPERIMENTAL

Animal material

The marine sponge *Siphonochalina siphonella* was collected from Sharm Obhur, Jeddah, at the Saudi Arabian Red Sea coast by Scuba divers in 2012. The sponge was deep-frozen immediately after collection and then freeze-dried to give the dry material.

General experimental procedures

The 1D-NMR and 2D-NMR spectra were recorded on a Bruker AMX-500 spectrometer with tetramethylsilane (TMS) as an internal standard. Thin layer chromatography (TLC) was performed on precoated silica gel F254 plates (E. Merck, Darmstadt, Germany). All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

For cytotoxicity assay, Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25 % Trypsin-EDTA were purchased from (Bio Whittaker ® Lonza, Belgium).

For cytotoxicity assay, crystal violet, composed of 0.5 % (w/v) crystal violet and 50 % methanol, was used as staining solution. The mammalian cell lines used: HepG2 cells (human cell line of a well differentiated hepatocellular carcinoma isolated from a liver biopsy of a male Caucasian aged 15 years) were obtained from the American Type Culture Collection (ATCC), while the MCF-7 cells (human breast cancer cell line) were obtained from VACSERA Tissue Culture Unit.

Two Gram-positive [*Staphylococcus aureus* (ATCC 25923), *Bacillis subtilis* (RCMB 010067) and two Gram-negative *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052)] bacteria were used for assessing the antimicrobial activity. The microbial strains were obtained from American Type Culture Collection (ATCC).

Extraction and isolation

The freeze dried sponge (95 g) was extracted by maceration with 70 % ethanol (3 × 1 L) at room temperature (around 23 °C). After filtration and evaporation of the solvent under vacuum, the combined ethanolic extract (30 gm) was suspended in water (200 mL) and successively partitioned with *n*-hexane (3 × 400 mL), dichloromethane (3 × 400 mL) and n-butanol (3 × 400 mL) to obtain the corresponding extracts.

Both *n*-hexane and dichloromethane extracts were pooled together based on their close similarity on TLC. The combined extracts were further purified by application on top of a silica gel packed column (Merck), eluted with *n*-hexane. Polarity was gradually increased with ethyl acetate to obtain six main fractions (A-F). Direct crystallization of fraction A eluted by 10 % ethyl acetate/*n*-hexane yielded compound 1 (350 mg).

Fractions D and E were separately purified by chromatotron (Harrison Research, Palo Alto, California, CA, USA) using 10 % ethyl acetate/*n*-hexane to give compound 2 (150 mg) and 3 (40 mg), while compound 4 (40 mg) was obtained by using 15 % $CH_3CN/CHCl_3$.

Cytotoxic activity assay

The cells were propagated in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum, 1 % L-glutamine, HEPES buffer and 50 µg/mL gentamicin. Cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2 and were sub-cultured two times a week. The cytotoxic activity was evaluated by the crystal violet staining (CVS) method [12]. Briefly, the cells were seeded in a 96-well tissue culture microplate, at a concentration of 1×10^4 cells per well in 100 µL of growth medium at 37 °C. After 24 h of seeding, fresh medium containing various concentrations of the tested compounds (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) were added to the microtiter plates (each compound was tested in triplicate for all concentrations). Next, the microtiter plates were incubated at 37 °C in a humidified incubator with 5 % CO_2. Control cell were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells was found not to affect the experiment.

After 48 h incubation period, viable cells yield was determined calorimetrically. In brief, the media were aspirated and the crystal violet solution (1 %) was added to each well for 30 min. The plates were rinsed after removing the stain by distilled water. Glacial acetic acid (30 %) was then added to all wells and mixed thoroughly. The evaluation of the fixed cells was performed calorimetrically by measuring absorbance at 595 mm in an automatic Microplate reader (TECAN, Inc.). The effect on cell growth was calculated as the presence and absence of the tested compounds. A dose-response curve was plotted to the concentration at which the growth of cells was inhibited to 50 % of the control (IC_{50}). The standard anti-tumor drug used was Doxorubicin®.

Antimicrobial assay

The anti-bacterial activity was evaluated using the agar-well diffusion method. Mueller-Hinton agar was used as media for bacteria. Bacterial suspension was prepared using the sterile saline water equivalent to a 0.5 McFarland standard and was cultivated on agar medium. Afterwards, 6 mm diameter wells were pressed in the used agar plates. Zone of inhibitions were compared with the standard drugs Ampicillin® and Gentamicin® (30 µg/ mL) against Gram-positive and Gram-negative bacteria, respectively. The bacterial plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured to evaluate the anti-microbial activity. Each test was performed twice and the average of the results was calculated. The extraction solvents were used as negative control [13].

Antiviral activity assay

The assay used is based on measuring the inhibition capacity of the tested samples on the virus-induced cytopathic effect (CPE) in susceptible mammalian cells (kidney epithelial cells of monkey) [14]. Monolayers of 10,000 vero cells, adhered at the bottom of a 96-well microtiter plate wells, were incubated with 5 % CO_2 for 24 h in a humidified incubator at 37 ^oC. The plates were washed with fresh Dulbecco's Modified Eagle's Medium (DMEM) and challenged with 10^4 Herpes simplex type II virus doses and instantaneously treated with two-fold serial dilutions of tested compounds in fresh medium. The plates were incubated at 37 ^oC for two days. At the same time, both an infection and an untreated vero cells controls, were conducted in the absence of the tested samples. The virus in the control wells was monitored every 24 h by an inverted microscope, until complete viral-induced cytopathic effect (CPE) was shown. The inhibition of cytopathic effect by the tested sample was used as a tool to measure their antiviral activity [15]. The monolayers were fixed with formalin and stained with a 0.1 % crystal violet solution. Three independent experiments were assessed each containing four replicates per treatment. Acyclovir® was used as a positive control under this assay system [16].

Statistical analysis

Results of the study were based on three independent experiments that were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Four-parameter logistic regression analysis was used to determine the medium inhibitory concentration at half-maximal (IC_{50}) of the compounds. Unpaired Student t-test was conducted on the results using GraphPad InStat (ISI Software) computer program. Differences were considered significant at $p < 0.05$.

RESULTS

Identification of triterpenes

The identification of the isolated triterpenes was based on extensive analyses of their HR-MS, 1D and 2D NMR (COSY, HSQC, HMBC) spectroscopic data and comparison with literature data. The structures of the isolated compounds are presented in Fig. 1.

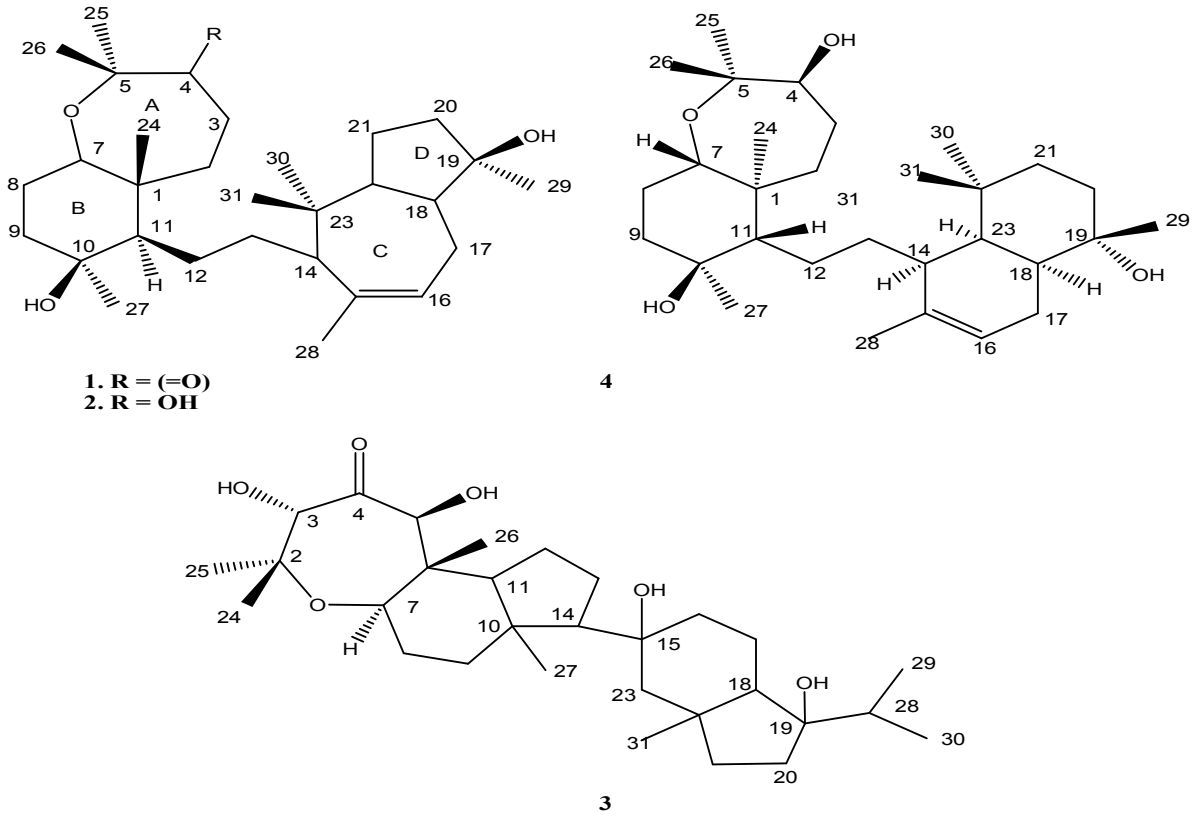

Fig 1: Structures of compounds 1 – 4

15 - sipholen-10, 19-diol - 4-one (Sipholenone A) (**1**). White crystals; m. p. 187 °C; HREIMS: *m/z* = 474.7156 (calc. for $C_{30}H_{50}O_4$, 474.37). ^{1}H δ ($CDCl_3$, 500 MHz); 0.84 (1H, *m*, H-11), 1.02 (3H, *s*, H-24), 1.09 (3H, *s*, H-30) 1.10 (3H, *s*, H-31), 1.15 (3H, *s*, H-29), 1.25 (3H, *s*, H-27), 1.24 (1H, *m*, H-2), 1.26 (3H, *s*, H-26), 1.30 (3H, *s*, H-25), 1.50 (1H, *m*, H-12), 1.52 (1H, *m*, H-9), 1.62 (1H, *m*, H-20), 1.68 (1H, *m*, H-8), 1.70 (1H, *m*, H-17), 1.73 (1H, *m*, H-21)1.76 (3H, br *s*, H-28), 1.77 (1H, *m*, H-18), 1.82 (1H, *m*, H-13) 1.83 (1H, *m*, H-2'), 1.93 (1H, *m*, H-13', H-21'), 2.10 (1H, *ddd*, J= 11.0, 6.3, 1.8 Hz, H-3), 2.11 (1H, *m*, H-14), 2.49 (1H, *m*, H-22), 2.91 (1H, *dd*, J= 11.5, 4.0 Hz, H-7), 3.21 (1H, *ddd*, J=13.0, 11.0, 2.4 Hz, H-3'), 5.45 (1H, br *dd*, J= 8.5, 5.0 Hz, H-16); ^{13}C-NMR δ ($CDCl_3$, 125 MHz): 12.2 (C-24), 20.5 (C-25), 24.8 (C-29), 25.1 (C-17), 26.5 (C-12), 26.7 (C-8), 29.5 (C-26), 30.0 (C-27), 30.1 (C-28), 31.2 (C-31), 31.6 (C-30), 33.8 (C-13), 35.1 (C-3), 35.5 (C-23), 37.3 (C-20), 39.2 (C-9), 39.7 (C-2), 42.1(C-1), 48.9 (C-18), 52.8 (C-22), 55.7 (C-11), 57.7 (C-14), 72.3 (C-10), 81.2 (C-7), 82.0 (C-19), 82.5 (C-5), 121.5 (C-16), 143.0 (C-15), 217.7 (C-4).

15-sipholen -4, 10, 19-triol (Sipholenol A) (**2**). White crystals; m. p. 188 °C; HREIMS: *m/z* = 476.4893 (calc. for $C_{30}H_{52}O_4$, 476.39). ^{1}H δ ($CDCl_3$, 500 MHz); 0.87 (1H, *m*, H-11), 1.00 (3H, *s*, H-24), 1.03 (3H, *s*, H-31), 1.08 (3H, *s*, H-30), 1.10 (3H, *s*, H-29), 1.13 (3H, *s*, H-26), 1.25 (3H, *s*, H-27), 1.27 (3H, *s*, H-25), 1.40 (1H, *m*, H-2), 1.50 (1H, *m*, H-2', 12), 1.54 (1H, *m*, H-9), 1.62 (1H, *m*, H-9'), 1.65 (1H, *m*, H-20), 1.71 (1H, *m*, H-8), 1.72 (1H, *m*, H-3), 1.73 (1H, *m*, H-17), 1.75 (1H, *m*, H-21), 1.76 (3H, br *s*, H-28), 1.79 (1H, *m*, H-13) 1.80 (1H, *m*, H-18), 1.97 (1H, *m*, H-3'), 1.98 (2H, *m*, H-13', H-21'), 2.02 (1H, *m*, H-14), 2.49 (1H, *m*, H-22), 3.46 (1H, *dd*, J= 11.0, 4.5 Hz, H-7), 3.50 (1H, *d*, J= 8.0 Hz, OH-5), 3.81 (1H, *dd*, J= 6.5, 3.5 Hz), 3.98 (1H, *d*, J=4.5 Hz, OH-3), 5.45 (1H, br *dd*, J= 8.5, 4.5 Hz, H-16); ^{13}C-NMR δ ($CDCl_3$, 125 MHz): 13.1 (C-24), 21.5 (C-25), 24.9 (C-17), 25.1 (C-3), 25.3 (C-21), 25.7 (C-29), 26.8 (C-8, C-12), 29.2 (C-26), 29.6 (C-27), 29.9 (C-30), 30.2 (C-28), 31.7 (C-31), 33.8 (C-13), 34.0 (C-2), 35.5 (C-23), 37.3 (C-20), 39.2 (C-9), 42.8 (C-1), 49.0 (C-18), 53.0 (C-22), 55.9 (C-11), 57.7 (C-14), 72.5 (C-10), 76.3 (C-7), 76.8 (C-4), 78.0 (C-5), 82.2 (C-19), 121.4 (C-16), 143.2 (C-15).

Neviotine A (**3**). White crystals; m.p. 214 °C; HREIMS: *m/z* = 506.7143 (calc. for $C_{30}H_{50}O_6$, 506.36). ^{1}H δ ($CDCl_3$, 500 MHz); 0.67 (1H, *m*, H-26), 0.87 (1H, *d*, J= 6.6, H-30), 0.89 (1H, *d*, J= 6.0, H-29), 1.17 (1H, *m*, H-25), 1.11 (1H, *m*, H-16), 1.25 (1H, *m*, H-31), 1.31 (1H, *m*, H-24), 1.35 (1H, *m*, H-17), 1.30 (1H, *m*, H-27), 1.38 (1H, *m*,

H-14), 1.41 (1H, *m*, H-18), 1.46 (1H, *m*, H-23), 1.45 (1H, *m*, H-17', 21), 1.46 (1H, *m*, H-23), 1.65 (1H, *m*, H-16'), 1.69 (1H, *m*, H-12), 1.70 (1H, *m*, H-13), 1.71 (1H, *m*, H-20), 1.76 (1H, *m*, H-13', 23,28), 1.76 (1H, *s*, 28), 1.82 *m* (1H, *m*, H-8, 20), 1.83 (1H, *m*, H-12'), 1.84 (1H, *m*, H-8'), 1.88 (1H, *m*, H-9), 1.54 (1H, *m*, H-11), 2.30 (1H, *m*, H-21), 3.50 (1H, *d*, J= 8.0 Hz, OH-5), 3.98 (1H, *d*, J= 4.5 Hz, OH-3), 4.16 (1H, *d*, J= 4.0 Hz, H-3), 4.92 (1H, *dd*, J= 13.0, 3.5 Hz, H-7), 5.03 (1H, *d*, J= 5.0 Hz, H-5); ^{13}C-NMR δ ($CDCl_3$, 125 MHz): 14.1 (C-26), 16.9 (C-30), 17.5 (29), 20.5 (C-27), 21.0 (C-17), 22.2 (C-13), 22.7 (C-12, 24), 26.5 (C-8), 26.9 (C-25), 31.6 (C-28), 33.1 (C31), 35.2 (C-21), 35.8 (C-20), 36.7 (C-9), 37.1 (C-16), 42.1 (C-22),43.3 (C-10), 44.4 (C-6), 47.4 (C-23), 54.8 (C-18), 55.8 (C-10), 62.4 (C-14), 68.9 (C-7), 74.5 (C-15), 75.7 (C-5), 76.8 (C-2), 84.3 (C-3), 88.3 (C-19), 212.9 (C-4).

Sipholenol L (**4**). White crystals; m.p. 107 °C; HREIMS: *m/z* = 476.4865 (calc. for $C_{30}H_{52}O_4$, 476.39). ^{1}H δ ($CDCl_3$, 500 MHz); 0.87 (1H, *s*, H-31), 0.90 (1H, *m*, H-11), 0.98 (1H, *s*, H-24), 1.04 (1H, *m*, H-21), 1.07 (1H, *s*, H-30), 1.10 (1H, *s*, H-25), 1.13 (1H, *s*, H-27), 1.15 (1H, *s*, H-29), 1.25 (1H, *s*, H-26), 1.30 (1H, *m*, H-20), 1.38 (1H, *m*, H-8), 1.44 (1H, *m*, H-2), 1.48 (1H, *m*, H-12), 1.52 (1H, *m*, H-9), 1.59 (1H, *m*, H-2'), 1.70 (1H, *m*, H-3), 1.72 (1H, *m*, H-8'), 1.73 (1H, *m*, H-21'), 1.74 (1H, *m*, H-20'), 1.75 (1H, *m*, H-13), 1.75 (1H, *s*, H-28), 1.80 (1H, *m*, H-17), 1.86 (1H, *m*, H-18), 1.99 (1H, *m*, H-3'), 2.05 (1H, *m*, H-23), 2.10 (1H, *m*, H-17), 2.44 (1H, br*s*, H-14), 3.49 (1H, *dd*, J= 12.0, 4.5 Hz, H-7), 3.80 (1H, *d*, J= 7.0 Hz, H-4), 5.26 (1H, br*s*, H-16); ^{13}C-NMR δ ($CDCl_3$, 125 MHz): 13.3 (C-24), 21.4 (C-26), 22.0 (C-28), 24.8 (C-31), 25.3 (C-3), 26.8 (C-8), 27.6 (C-12), 28.6 (C-29), 29.2 (C-17, 25), 30.5 (C-20, 27), 34.4 (C-2), 34.9 (C-22), 35.6 (C-30), 36.4 (C-13), 39.3 (C-9), 39.8 (C-21), 42.1 (C-23), 42.9 (C-1), 47.2 (C-14), 47.7 (C-18), 56.7 (C-11), 72.4 (C-10), 73.0 (C-19), 76.8 (C-7), 77.0 (C-4), 77.9 (C-5), 121.8 (C-16), 135.7 (C-15).

Cytotoxic activity

Results of cytotoxicity are presented in Tables 1 and 2. All four compounds possessed a dose dependent cytotoxic effect against the two tested cell lines. However, compound **1** (sipholenone A) showed the highest cytotoxic activities against both MCF-7 and HepG-2 cell lines (IC_{50} = 3.0 ± 0.4 µg/ml and 2.8 ± 0.4 µg/ml, respectively). Compound **4** (sipholenol L) was significantly ($p < 0.05$) more selective on MCF-7 (IC_{50} = 4.0 ± 0.2 µg/ml) than on the HepG-2 cell line (IC_{50} = 18.7 ± 0.9 µg/mL) compared to doxorubicine. On the other hand, compound **2** (sipholenol A) was more cytotoxic against HepG-2 (IC_{50} = 9.6 ± 0.8 µg/mL) than the MCF-7 (IC_{50} = 19.2 ± 0.6 µg/mL) cell line. Finally, compound **3** showed moderate cytotoxic activity against both tested cell lines (IC_{50} = 12.3 ± 0.7 µg/ml and 11.8 ± 1.2 against MCF-7 and Hep-G, respectively).

Antimicrobial activity

In the current study, the anti-microbial activity of the isolated compounds was evaluated by determining the zone of inhibition against two Gram-positive and two Gram-negative bacteria. Results of antimicrobial activity are presented in Table 3. Compound **3** exhibited the highest antimicrobial activity (12.7 ± 0.58 - 17.2 ± 0.58 mm, while compound **4** revealed moderate to low activity against Gram positive bacteria only (12.3 ± 0.72 - 14.5 ± 0.72 mm. Compound **2** was totally ineffective against all tested microorganisms at 20 mg/mL. Furthermore, the Gram-negative bacteria *Pseudomonas aeruginosa* was resistant to all compounds. The anti-microbial activity of each of tested compounds is less than that of the standard. Therefore, no minimum inhibitory concentration was determined.

Table 1: Cytotoxicity of compounds **1** - **4** against MCF-7 cell line

Tumor cell line MCF-7		**Mean of surviving fraction (± SD)[#]**			
Sample concentration (µg/mL)	***Doxorubicin***	***1***	***2***	***3***	***4***
50	3.24 ± 1.00	4.98 ± 0.32	17.81±1.84	22.96±1.36	16.73±2.31
25	6.55 ± 1.20	8.64 ± 0.78	38.82±2.87	36.73±2.05	23.41±1.25
12.5	11.74 ± 2.20	15.82±0.96	63.11±3.27	49.34±1.86	29.98±0.86
6.25	17.22 ± 2.00	26.47±0.63	87.18±0.86	68.22±1.75	36.58±2.34
3.125	21.18 ± 2.70	48.02±2.38	94.86±0.24	83.74±0.86	54.97±0.98
1.56	30.86 ± 2.90	81.53±2.89	98.14±0.18	94.85±0.31	73.16±1.24
*IC_{50} µg/mL	0.43±0.20	3.00±0.40	19.20±0.60	12.30±0.70	4.00±0.20

*[#]Mean of surviving fraction ± standard deviation: Mean of three assays. $p < 0.05$ compared to reference drug; *IC_{50}: concentration required to reduce cell survival by 50 %*

Table 2: Cytotoxicity of compounds **1** - **4** against HepG-2 cell line

Tumor cell line HepG-2	**Mean of surviving fraction (± SD)** [#]				
Sample concentration (µg/mL)	***Doxorubicin***	***1***	***2***	***3***	***4***
50	6.82±1.40	4.25±0.21	8.04±0.16	15.48±1.32	13.17±1.89
25	8.89±2.20	9.77±0.35	17.47±1.97	22.06±2.98	39.58±2.44
12.5	14.83±2.30	13.59±0.41	36.72±2.86	47.67±2.87	60.19±3.07
6.25	16.16±3.20	21.68±0.22	65.51±3.47	68.52±1.86	71.08±0.89
3.13	22.28±2.00	42.16±1.86	79.49±0.86	85.11±0.26	78.86±0.21
1.56	34.64±1.90	78.42±2.09	91.18±0.34	94.23±0.35	83.04±0.14
*IC_{50} µg/mL	0.469±0.60	2.80±0.40	9.60±0.80	11.80±1.20	18.70±0.90

[#]*Mean of surviving fraction ± standard deviation: Mean of three assays; p <0.05 compared to reference drug.*
**IC*$_{50}$*: concentration required to reduce cell survival by 50 %*

Antiviral activity

All four *S. siphonella* triterpenes were tested against two viruses, the hepatitis A virus (HAV-10) and the herpes simplex virus (HSV-1). The results showed moderate anti-viral activity for compound **2** against HAV-10 virus, whereas compound **4** was weakly active against both viruses. Compound **3** showed weak activity against HAV-10 virus only.

DISCUSSION

Compound **1** was obtained as white crystals. It displayed a m/z of 474.7156 suggesting a molecular formula $C_{30}H_{50}O_4$ with 6 degrees of unsaturation. Its ^{13}C-NMR and DEPT spectrum exhibited 30 carbons, including eight singlet methyl signals, nine methylenes, six methines one of which is olefinic and seven quaternary carbons. Moreover, the presence of a double bond at δ_H 5.45 and δ_C 143.0, 121.5 and a ketonic group (δ_C 213.4) in the NMR data suggested a tetracyclic structure. Comparing the above data with those of the previously isolated compounds from *Siphonochalina* revealed the basic skeleton of squalene-derived cyclic ether triterpene. These data confirm the structure of compound **1** to be sipholenone A [6,9,10].

Compound **2** was also isolated as white crystals and analyzed for $C_{30}H_{52}O_4$. The NMR data were identical with those of sipholenone (compound **1**), except for the absence of the carbonyl carbon which is replaced by oxymethine group at C-4 (δ_H 3.81, dd 6.5, 3.5) and δ_C (76.8). The MS and NMR spectral data confirmed compound **2** to be sipholenol A [3,6,9,10].

Compound **3** was also isolated as white crystals. HRMS displayed a molecular ion peak at m/z of 506.7143 suggesting a molecular formula $C_{30}H_{50}O_6$ with 6 degrees of unsaturation. DEPT experiments of 3 revealed the presence of seven methyl groups (rather than eight in the sipholanes), two of which were doublets (δ_H 0.89 and 0.87, J = 6.6), nine methylenes, seven methines and seven quaternary carbons which account together for thirty carbon atoms and indicating a neviotane triterpenoidal skeleton rather than sipholane-type. Both H-3 (δ_H 4.16) and H-5 (δ_H 5.03) appeared as doublets with coupling constants of 4.0 and 8.0 Hz consequently, due to coupling with the hydroxyl groups attached at the same positions.

Table 3: Antimicrobial activity of triterpenoid constituents (compounds **1** – **4**) isolated from the sponge *S. siphonella* expressed as diameters of inhibition zone (mm)

Tested microorganisms	**Mean zone of inhibition in mm ± S.D.**				
	1	**2**	**3**	**4**	*Ampicillin*
Gram Positive Bacteria					
Staphylococcus aureus (RCMB 010028)	8.2±0.72	NA	14.1±0.72	12.3±0.72	27.4±0.18
Bacillis subtilis (RCMB 010067)	2.4±0.58	NA	17.2±0.58	14.5±0.72	32.4±0.10
Gram Negative Bacteria					Gentamicin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	17.3±0.15
Escherichia coli (RCMB 010052)	5.4±0.58	NA	12.7±0.58	NA	22.3±0.18

**data are expressed as mean ± SD); diameter of the inhibition zone (mm) beyond the well diameter of 6 mm; NA = no activity*

Comparing the MS and NMR spectral data of 3 with those reported for neviotine A, previously isolated from *S. siphonella* [8,17] suggested that both compounds are identical. This is the third report for isolation of neviotine A from *S. siphonella* sponge, or elsewhere, which indicates the chemical variability of the triterpenoidal constituents of this sponge from different areas of the Red Sea [3].

Compound **4**, isolated as white crystals, displayed a molecular ion peak at m/z of 476.4865 calculated for $C_{30}H_{52}O_4$. The NMR data revealed a squalene-type triterpene with rings A and B exactly similar to those of compounds **1** and **2**, while rings C and D were fused to form a cis-decaline system. Compound **4** was identified as sipholenol L, by comparing its physical and spectral data including MS, ^{1}H NMR and ^{13}C NMR data with those reported for sipholenone L [18].

From the results shown above, it could be concluded that all compounds possessed a dose dependent cytotoxic effect against the two tested cell lines. However, sipholenone A (compound **1**) showed the highest cytotoxic activities against both MCF-7 and HepG-2 tumor cell lines (IC_{50} = 3.0 and 2.8 µg/mL, respectively), while sipholenol L (compound 4) was significantly active against MCF-7 cell line (IC_{50} = 4.0 µg/mL). Thus, both sipholenone A and sipholenol L could be potential candidates to develop promising drugs to treat cancer.

This is the first report for anti-microbial evaluation of neviotane-type triterpenes. A previously published study by Gab-Alla *et al*, revealed that the total methanolic extract of *S. siphonella* was inactive against some tested gram positive and gram negative bacterial strains [19]. However, the results of the present study are partly in agreement with those published by Aqil *et al* which showed that sipholenone A and sipholenol A are inactive against the Gram-positive bacteria *S. aureus* and *B. subtilis*; and the Gram-negative bacteria *P. aeruginosa* and *E. coli* [20].

CONCLUSION

Four sipholane and neviotane-type triterpenes have been isolated and identified from the Red Sea sponge *S. siphonella*. Compound 1 has significant cytotoxic activity against MCF-7 and HepG-2.

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