

Original Research Article

Antibacterial and cytotoxic properties of isoprenoids from the red sea soft coral, *Lobophytum* sp

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Abstract

Purpose: To evaluate the antibacterial and cytotoxic activities of the secondary metabolites of *Lobophytum* sp.

Methods: Maceration with methanol: chloroform (1:1) was applied to extract the coral material. Chromatographic and spectroscopic techniques were employed for fractionation, isolation and elucidation of pure compounds. Antibacterial activities were performed by well diffusion method against three Gram-positive and four Gram-negative bacteria. Brine shrimp lethality test was employed to predict toxicity, while antitumor activity were tested by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method against Ehrlich carcinoma cells.

Results: Four sesquiterpenes, one cembranoid type diterpenes and two steroids were isolated. **1** exhibited significant antibacterial activity against four tested bacteria (*P. aeruginosa*, *S. aureus*, *S. epidermis*, and *S. pneumonia*) with MIC value of 15 µg/mL. Moreover, **1** showed high diameter zone of inhibition ranging from 16 - 18 mm against test bacteria. Compounds **4** and **5** displayed moderate antibacterial activity against all test bacteria with inhibition zone diameter (IZD) ranging from 11 - 15 mm and MIC values of 30 µg/mL. **2**, **3**, **6** and **7** exhibited weak antibacterial activity (IZD, 7 - 11 mm; MIC ≥ 30 µg/mL). In addition, only diterpene compound (**4**) showed high toxicity against A. Salina and antitumor activity against Ehrlich carcinoma cells with the LD₅₀ of 25 and 50 µg/mL, respectively.

Conclusion: This study reveals the strong antibacterial activity of sesquiterpene alismol (**1**) and the potential antibacterial and antitumor activity of cembranoid type diterpene, cembrene A (**4**).

Keywords: Soft coral, *Lobophytum* sp., Red Sea, Antibacterial, Cytotoxicity, Sesquiterpene Alismol, Cembranoid, Diterpene, Cembrene

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INTRODUCTION

Marine soft corals of the subclass *Alcyonaria* (Octocorallia) have fascinated a great attention in the light of the structural diversity and broad range of biological activities of their metabolites, such as sesquiterpenoids, diterpenoids, tetraterpenoids and steroids [1]. In a continuing

efforts at studying marine metabolites, collected from Red Sea Saudi Arabia, with biological activity, we have previously isolated several bioactive secondary metabolites from *Sarcophyton trocheliophorum* and *S. glaucum* [2-5] Recently, our interest in alcyonaria coral, led us to isolate several secondary metabolites from *Lobophytum* sp. Literature survey showed that

metabolites of *Lobophytum* sp exhibited various biological activities, such as antitumor [6-13], antibacterial [14], anti-inflammatory activities [14-16] and antiviral [17]. Our investigation led to the isolation and characterization of four sesquiterpenes: alismol (**1**), nardol (**2**), aristol-9-ene (**3**), alismoxide (**5**), one cembranoid type diterpene, cembrene A (**4**), and two steroid type compounds, Chalinasterol (**6**) and Nephasterol C (**7**). To the best of our knowledge, this is the first report of the presence of sesquiterpenes from soft coral *Lobophytum* sp. Antibacterial and antitumor activities of all metabolites were also evaluated. This paper describes isolation, NMR structural elucidation and biological activity of the following compounds.

EXPERIMENTAL

Animal material

The soft coral, *Lobophytum* sp., was collected in May, 2014, off the Saudi Arabia Red Sea Coast at Jeddah and identified by Dr. Mohsen El-Sherbiny (Marine Biology Department, Faculty of Marine Sciences, King Abdulaziz University). Voucher sample (no. JAD 09063B) was deposited in the herbarium of the Marine Biology Department, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

General experimental procedures

Chromatographic material: Silica gel 60 for column chromatography (60–120 mesh LR), for thin-layer chromatography. TLC aluminum sheets 20 x 20 cm. Silica gel 60 F254 was used

for preparative, Pre-coated TLC glass plates SIL G-25 UV254, 0.25 mm silica gel (E. Merck, Darmstadt, Germany). Nuclear Magnetic Resonance spectra were recorded for 1D- and 2D-NMR: Bruker AVANCE III WM at 600 MHz and ^{13}C -NMR at 150 MHz spectrometer. Chemical shifts are given δ (ppm) relative to TMS as internal standard. Electron impact mass spectra were determined at 70 eV using a Kratos GCMS-25 instrument. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO. USA).

Bacterial isolates

Seven bacteria were obtained from King Fahd General Hospital, Jeddah, Saudi Arabia; they were *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumonia* (Gram-positive bacteria), *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* spp (Gram-negative bacteria), Brine shrimp *Artemia salina*, and Ehrlich carcinoma cells were from the culture collection of Microbiology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

Extraction and isolation

Coral material was washed with water and dried in the shade at room temperature. The dried material (90 g) was exhaustively extracted by maceration method with equal volumes of $\text{CHCl}_3/\text{MeOH}$ (2 x 6 l, 24 h for each batch) at room temperature.

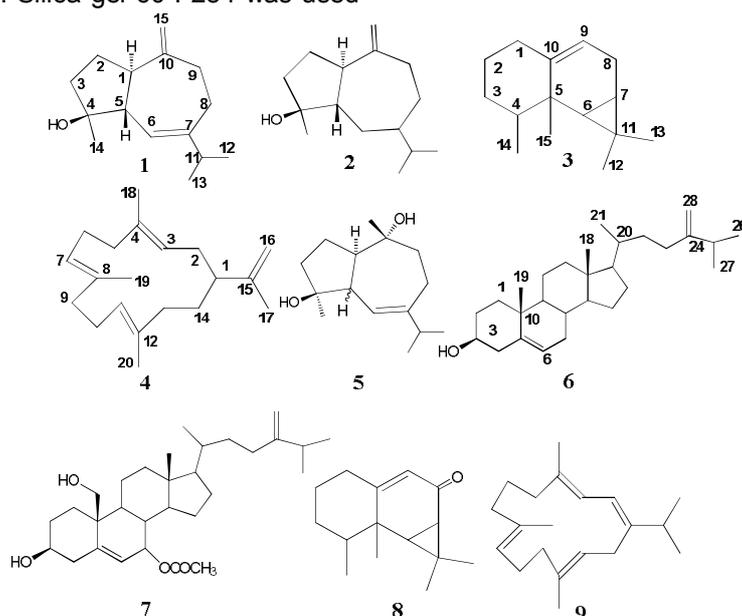


Figure 1: Chemical structures of 1 - 9

The residue (20 g) was chromatographed on NP (Merck, 60G) column chromatography employing n-hexane/methylene chloride, followed by ethyl acetate/methanol mixtures with increasing polarity. Fractions of ~100 mL were collected. TLC was carried out by employing silica gel chromatoplates, appropriate solvent system, and 50 %-sulfuric acid in methanol as spraying reagent. Fractions containing a single compound were combined and further purified by preparative TLC of glass supported silica gel plates (20 x 20 cm) of 250 μ m thickness.

The fraction 1 eluted with n-hexane - dichloromethane (9:1, 37.00 mg) was purified by preparative TLC using solvent system n-hexane: ethyl acetate (9:1) to give two bands. The first band with $R_f = 0.96$ (brown color with sulfuric acid-methanol) was taken to give compound 3 as crystalline (5.0 mg). The second band with $R_f = 0.36$ (black color with sulfuric acid-methanol) was taken to give compound 4 as colorless oil (4.8 mg). The fraction 2 eluted with n-hexane - dichloromethane (7:3, 35.00 mg) was purified by preparative TLC using the solvent system n-hexane:ethyl acetate (8:2) to give two bands. The first band with $R_f = 0.48$ (broad purple color after spraying with sulfuric acid-methanol) was taken to give crystalline of compound 1 (6.5 mg). The second band with $R_f = 0.30$ (purple color with sulfuric acid-methanol) was taken to give colorless oil of compound 2 (4.6 mg). The fraction 3 eluted with n-hexane - dichloromethane (5:5, 40.00 mg) was purified by preparative TLC using solvent system n-hexane: ethyl acetate (7:3) to give one band with $R_f = 0.72$ (brown color with sulfuric acid-methanol) was taken to give compound 6 as crystalline (5.0 mg). The fraction 4 eluted with dichloromethane:ethyl acetate (9:1, 42.00 mg) was purified by preparative TLC using the solvent system n-hexane : ethyl acetate (6:4) to give one band with $R_f = 0.38$ (broad purple color after spraying with sulfuric acid-methanol) was taken to give crystalline of compound 5 (8.0 mg). The fraction 5 eluted with dichloromethane:ethyl acetate (8:2, 41.00 mg) was purified by preparative TLC using the solvent system n-hexane:ethyl acetate (5:5) to give one band with $R_f = 0.62$ (brown color after spraying with sulfuric acid-methanol) was taken to give powder of compound 7 (4.2 mg).

Antibacterial activity assay

The growth inhibition of the isolated compounds against several pathogenic bacteria was carried out using agar well diffusion method as described by Holder and Boyce (1994) [18]. 0.1 mL of suspended bacterium in sterile medium

(1.5×10^8 CFU/mL) was spread on Mueller-Hinton agar. 50 μ L of each sample (10 μ g/mL) were poured to the wells (6-mm diameter). All plates were left for 1 h at 48 °C and then incubated for 24 h at 37 °C. Inhibition zone diameters formed around the well were measured and mean diameter of three replica was calculated. DMSO was used as a negative control and ampicillin as a positive control. Minimum inhibitory concentrations (MICs) were determined by modified method described by Chand *et al* [19] and Aly and Gumgumji [20]. 175 μ L of an exponentially growing culture ($\times 10^6$ – 10^7 CFU/mL) was delivered into each well of microtiter plate along with 20 μ L solution of each concentration of the isolated compounds. The appropriate solvent was used as control. After incubation for 40 min., 5 μ L of a solution of Fluorescein diacetate (FDA) 0.2 % (w/v) in acetone was added into microtiter plate, then the incubation was continued for 90 min. The green color produced from the hydrolysis of FDA was measured at 490 nm (MR7000 automatic ELISA tray reader) along with the blanked wells and control wells containing microbial cultures.

Toxicity activity assay

The cytotoxicity of the isolated compounds can be measured by using brine shrimp lethality test [21]. Isolated compounds were dissolved in DMSO at varying concentrations and then incubated with the brine shrimp larvae in sea water. Brine shrimp larvae as positive control and DMSO as negative control were also incubated. The average number of larvae that survived in each vial was determined after 24 h. The mean of mortality percentage was plotted against the logarithm of concentrations. The concentration that killed fifty percent of the larvae (LC_{50}) was determined from the graph.

Antitumor activity assay

Ehrlich carcinoma cell lines was used to determine the antitumor activity of the isolated compounds. The cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, USA) with 10 % fetal calf serum (Gibco, USA) at 37 °C under a humidified atmosphere (95 % air and 5 % CO_2) for 48 h. Cells were treated with different concentration of the isolated compounds (200–1,000 μ L/mL) for 24 h, centrifuged for 2 min at 1,500 g and counted under hemacytometer after removing the supernatant using trypan blue (Sigma, USA) in normal saline (1:1 v/v). The percentage of cell viability was assessed to determine the 50 % lethal dose by which 50 % of cells are killed (LD_{50}).

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean \pm standard deviation (SD). Unpaired student t-test was carried out to detect any significant differences between the results of control and the treated samples using GraphPad InStat (ISI Software). Differences were considered significant at $p < 0.05$.

RESULTS

Compounds

Identification of secondary metabolites was analyzed based on MS and 1D/2D NMR data and comparison with the literature. The chemical structure of isolated compounds can be seen in the Fig. 1.

Alismol (1): Colorless oil; HRESIMS: $m/z=220.3505$ (Calc. for $C_{15}H_{24}O$, 220.1820); 1H NMR (600 MHz, $CDCl_3$): δ_H 5.55 (1H, br s, H-6), 4.76 (1H, s, Ha-15), 4.70 (1H, s, Hb-15), 2.28 (1H, dd, $J=14.4, 7.4$ Hz, H-5), 2.50 (dd, $J=21.6, 10.2$ Hz, H₁-1), 2.06 (dd, $J=38.4, 10.2$ Hz, H₂-1), 2.30 (1H, d, $J=7.4$ Hz, H-1), 2.24 (1H, m, H-11), 2.20 (1H, m, Ha-8), 2.10 (1H, m, Hb-8), 1.90 (1H, m, H-2), 1.74 (2H, m, H-3), 1.72 (1H, m, H-2'), 1.25 (3H, s, H-14), 1.01 (3H, d, $J=6.6$ Hz, H-12), 0.98 (3H, d, $J=6.6$ Hz, H-13); ^{13}C NMR (200 MHz, $CDCl_3$): δ_C 153.93 (C-10), 149.78 (C-7), 121.30 (C-6), 106.51 (C-15), 80.69 (C-4), 55.01 (C-1), 47.27 (C-5), 40.25 (C-3), 37.44 (C-11), 37.08 (C-9), 30.00 (C-8), 24.76 (C-2), 24.09 (C-14), 21.48 (C-13), 21.30 (C-12).

Nardol (2): Colorless Oil; HRESIMS: $m/z=222.3663$ (Calc. for $C_{15}H_{26}O$, 222.1984); 1H NMR (600 MHz, $CDCl_3$): δ_H 4.73 (1H, s, H-15'), 4.71 (1H, s, H-15), 2.89 (1H, d, H-1), 2.40 (1H, m, H-9), 2.30 (1H, m, H-7), 2.16 (1H, m, H-8), 2.14 (1H, m, H-9'), 1.94 (1H, m, H-6), 1.84 (1H, m, H-3), 1.72 (1H, m, H-6'), 1.71 (1H, m, H-5), 1.62 (1H, m, H-3'), 1.58 (1H, m, H-11), 1.52 (1H, m, H-8'), 1.44 (3H, s, H-14), 1.30 (2H, m, H-2), 1.01 (3H, d, $J=6.6$ Hz, H-12), 0.96 (3H, d, $J=6.6$ Hz, H-13); ^{13}C NMR (200 MHz, $CDCl_3$): δ_C 151.63 (C-10), 107.28 (C-15), 80.31 (C-4), 62.18 (C-1), 57.57 (C-5), 47.91 (C-7), 41.50 (C-3), 34.58 (C-11), 34.06 (C-9), 29.15 (C-8), 26.27 (C-6), 24.95 (C-14), 22.71 (C-2), 18.72 (C-13), 17.74 (C-12).

Aristol-9-ene (3): Colorless oil; HRESIMS: $m/z=204.3511$ (Calc. for $C_{15}H_{24}$, 204.1878); 1H NMR (600 MHz, $CDCl_3$): δ_H 5.10 (1H, ddd, 2.4, 2.4, 4.2 Hz, H-9), 2.16 (1H, m, H-1), 1.98 (1H, m, H-1), 2.08 (1H, dd, 2.4, 4.2, Ha-8), 2.04 (1H, dd, 2.4, 4.2, Hb-8), 1.69 (1H, dd, 1.2, 2.4, H-4), 1.62 (H,

m, Ha-3), 1.42 (1H, m, Ha-2), 1.36 (1H, m, Hb-3), 1.3 (1H, m, Hb-2), 1.18 (3H, s, H-13), 1.12 (3H, s, H-12), 1.05 (3H, s, H-15), 0.98 (3H, d, $J=4.8$ Hz, H-14), 0.75 (1H, dd, $J=6, 8.5$ Hz, H-7), 0.60 (1H, d, $J=8.5$ Hz, H-6); ^{13}C nmr (200 MHz, $CDCl_3$): δ_C 141.30 (C-10), 118.5 (C-9), 37.86 (C-4), 36.82 (C-5), 33.04 (C-1), 32.09 (C-6), 31.32 (C-2), 28.44 (C-7), 27.21 (C-3), 21.77 (C-8), 21.29 (C-13), 19.29 (C-14), 17.9 (C-11), 15.91 (C-12), 15.65 (C-15).

Cembrene A (4): Colorless oil, HRESIMS: $m/z=272.4681$ (Calc. for $C_{20}H_{32}$, 272.2504); 1H NMR (600 MHz, $CDCl_3$): δ_H 5.31 (1H, dd, $J=6.8, 15.3$ Hz, H-7), 5.25 (1H, dt, $J=1.7, 6.8, 8.5$ Hz, H-3), 5.08 (1H, dt, $J=6.0, 12.0$, H-11), 4.82 (2H, d, $J=1.0$, H-16), 2.26, (1H, m, Ha-6), 2.21 (1H, dddd, $J=3.4, 4.25, 10.2, 13.6$ Hz, H-1), 2.17 (1H, m, Ha-2), 2.15 (2H, m, H-10), 2.15 (1H, m, Ha-5), 2.13 (1H, m, Hb-6), 2.12 (1H, m, Ha-13), 2.09 (2H, m, H-9), 2.02 (1H, m, Hb-2), 1.96 (1H, m, Hb-5), 1.93 (1H, m, Hb-13), 1.79 (dq, $J=10.2; 13.6$ Hz, Ha-14), 1.41 (m, Hb-14), 1.63 (3H, s, H-17), 1.60 (3H, s, H-18), 1.55 (3H, s, H-19), 1.54 (3H, s, H-20); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 148.87 (C-15), 134.55 (C-8), 133.48 (C-4), 133.10 (C-12), 126.08 (C-11), 124.19 (C-7), 121.91 (C-3), 110.44 (C-16), 46.17 (C-1), 39.49 (C-9), 39.01 (C-5), 33.99 (C-13), 32.64 (C-2), 28.19 (C-14), 24.98 (C-6), 23.85 (C-10), 18.99 (C-17), 17.97 (C-18), 15.5 (C-19), 15.28 (C-20).

Alismoxide (5): Colorless oil, HRESIMS: $m/z=238.3657$ (Calc. for $C_{15}H_{26}O_2$, 238.1930), 1H NMR (600 MHz, $CDCl_3$): δ_H 5.50 (1H, dd, $J=2.0; 1.2$ Hz, H-6), 2.24 (1H, sept, H-11), 2.22 (1H, m, Ha-8), 2.18 (1H, m, H-5), 1.95 (1H, dd, 10.4; 1.2 Hz, Hb-8), 1.89 (1H, m, H-1), 1.80 (1H, m, Ha-2), 1.80 (1H, m, Ha-9), 1.70 (1H, m, Ha-3), 1.68 (1H, m, Hb-2), 1.62 (1H, m, Hb-3), 1.47 (1H, m, Hb-9), 1.27 (3H, s, H-14), 1.21 (3H, s, H-15), 0.99 (3H, d, $J=6.8$ Hz, H-12), 0.98 (3H, $J=6.8$ Hz, H-13); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 149.69 (C-7), 121.33 (C-6), 80.25 (C-4), 75.25 (C-10), 50.77 (C-1), 50.38 (C-5), 42.67 (C-9), 40.52 (C-3), 37.33 (C-11), 25.12 (C-8), 21.44 (C-14), 21.55 (C-2), 22.61 (C-15), 21.20 (C-13), 21.42 (C-12).

Chalinasterol (6): Amorphous powder, HRESIMS: $m/z=398.6642$ (Calc. for $C_{28}H_{46}O$, 398.3540), 1H NMR (600 MHz, $CDCl_3$): δ_H 5.38 (1H, s, H-6), 4.71 (1H, s, Ha-28), 4.65 (1H, s, Hb-28), 3.6 (1H, m, H-3), 1.03 (3H, d, $J=6.8$ Hz, H-27), 1.02 (3H, d, $J=6.8$ Hz, H-26'), 1.01 (3H, s, H-19), 0.98 (3H, d, $J=6.8$ Hz, H-26), 0.94 (3H, d, $J=6.8$, H-21), 0.68 (3H, s, H-18). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 156.98 (C-24), 140.79 (C-5), 121.73 (C-6), 105.91 (C-28), 71.83 (C-3), 56.77 (C-14), 55.99 (C-17), 50.13 (C-9), 42.37 (C-13), 42.31 (C-4), 39.79 (C-12), 37.48 (C-1), 36.51 (C-

10), 35.7 (C-20), 34.69 (C-23), 33.81 (C-25), 38.58 (C-8), 32.0 (C-7), 31.68 (C-2), 30.98 (C-22), 28.23 (C-16), 24.3 (C-15), 22.01 (C-27), 21.88 (C-26), 21.10 (C-11), 19.42 (C-19), 18.72 (C-21), 11.88 (C-18).

Nephalsterol C (7): Amorphous powder, HRESIMS: $m/z = 472.6997$ (Calc. for $C_{30}H_{48}O_4$, 472.3550), 1H NMR (600 MHz, $CDCl_3$): δ_H 5.58 (1H, t, $J = 2$ Hz, H-6), 4.90 (1H, dd, $J = 2.5, 6$ Hz, H-7), 4.71 (1H, d, $J = 0.85$ Hz, Ha-28), 4.65 (1H, d, $J = 0.85$ Hz, Hb-28), 3.80 (1H, d, $J = 11.9$ Hz, H-19), 3.65 (1H, d, $J = 11.9$ Hz, H-19'), 3.60 (1H, tt, $J = 10.2, 5.1$ Hz, H-3), 2.38 (1H, dddd, $J = 1.7, 2.5, 4.25, 5.95$ Hz, H-4), 2.20 (1H, tt, 6.8, 12.75, H-4'), 2.02 (3H, s, OAc), 1.02 (3H, d, $J = 5.1$ Hz, H-26), 1.01 (3H, d, $J = 5.1$ Hz, H-27), 0.94 (3H, d, $J = 5.9$ Hz, H-21), 0.74 (3H, s, H-18). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 171.38 (MeCOO-), 156.81 (C-24), 140.02 (C-5), 126.78 (C-6), 106.02 (C-28), 75.11 (C-7), 70.92 (C-3), 62.9 (C-19), 56.33 (C-14), 55.27 (C-17), 48.53 (C-9), 42.6 (C-13), 41.63 (C-4), 41.45 (C-10), 39.74 (C-12), 35.65 (C-8), 37.84 (C-20), 34.65 (C-22), 33.78 (C-25), 33.20 (C-1), 31.80 (C-2), 30.95 (C-23), 28.39 (C-16), 24.98 (C-15), 21.73 (C-26), 21.86 (C-27), 21.7 (CH₃COO), 22.01 (C-11), 18.74 (C-21), 12.13 (C-18).

Antibacterial activity

Antibacterial activity assay were performed against several bacteria. The result of diameter of zone of inhibitions (mm) of isolated compounds were presented in Table 1. There were significant differences ($p < 0.05$) among antibacterial activities of all isolated compounds. Compound **1** has significant antibacterial activity ($p < 0.05$) against both gram-positive bacteria (*S. aureus*, *S. S. epidermis* and *S. pneumonia*) and

gram-negative bacteria (*P. aeruginosa*) with the MIC value of 15 $\mu\text{g/mL}$ (Table 2). Moreover, compound **1** showed the larger diameter of inhibition zone ranging from 16-18 mm against those four bacteria. The diameter of inhibition zone against *S. epidermis* was recorded to be largest (18 ± 2.0 mm). Meanwhile, compound **4** and **5** exhibited moderate antibacterial activity with the diameter of inhibition zone ranging from 11 – 15 mm and MIC value of 30 $\mu\text{g/mL}$. Compound **2**, **3**, **6** and **7** showed weak antibacterial activity with diameter of inhibition zone ranging from 7 – 10 mm and MIC value ≥ 30 $\mu\text{g/mL}$.

Toxicity and antitumor activity

The result of toxicity and antitumor activity test can be seen in Table 3. All compounds did not show appreciable toxicity and antitumor properties against *Artemia salina* and Erhlich carcinoma cells, except for cembrene A (compound **4**). It exhibited significant toxicity and antitumor activity ($p < 0.05$) with the LD₅₀ values of 25 and 50 $\mu\text{g/mL}$, respectively.

DISCUSSION

Compound **1** was isolated as colorless oil. HRESIMS at m/z of 220.3505 and ^{13}C NMR suggesting a molecular formula of $C_{15}H_{24}O$ with 4 degrees of unsaturation. The obtained spectroscopic data (*cf. exp.*) was found to be identical with the structural data of alismol, a guaiane sesquiterpene isolated previously from the rhizome of *Alisma plantago-aquatu* L var *orientale* Samuelsson (Altsmataceae) [22,23].

Table 1: Antibacterial activity of compounds 1-7

Test bacteria	Mean diameter of the inhibition zone (mm) \pm SD*							Ampicillin ⁺
	1	2	3	4	5	6	7	
<i>Acinetobacter</i> spp	14 \pm 0.23	7 \pm 0.0	10 \pm 0.22	14 \pm 0.24	14 \pm 1.3	9 \pm 0.0	7 \pm 0.3	14 \pm 0.14
<i>Escherichia coli</i>	12 \pm 0.12	7 \pm 0.0	10 \pm 0.22	13 \pm 0.34	15 \pm 2.0	7 \pm 0.0	7 \pm 1.0	16 \pm 0.42
<i>Klebsiella pneumonia</i>	14 \pm 1.24	9 \pm 0.14	10 \pm 0.14	13 \pm 0.14	12 \pm 1.3	10 \pm 0.0	7 \pm 1.0	16 \pm 0.33
<i>Pseudomonas aeruginosa</i>	17 \pm 0.4	7 \pm 0.00	7 \pm 0.00	13 \pm 0.22	11 \pm 1.1	7 \pm 0.0	7 \pm 0.0	18 \pm 0.24
<i>Staphylococcus aureus</i>	16 \pm 0.22	7 \pm 0.0	7 \pm 0.00	11 \pm 0.33	13 \pm 2.0	7 \pm 0.0	9 \pm 1.0	26 \pm 0.22
<i>Staphylococcus epidermidis</i>	18 \pm 2.0	7 \pm 0.0	7 \pm 0.8	11 \pm 0.22	14 \pm 2.6	10 \pm 1.3	7 \pm 1.0	34 \pm 4.20
<i>Streptococcus pneumonia</i>	16 \pm 0.8	7 \pm 0.0	10 \pm 0.2	11 \pm 0.19	15 \pm 3.1	10 \pm 1.3	7 \pm 1.4	34 \pm 1.40

*Ampicillin used as control positive, *: Significant difference was recorded between all the tested value and ampicillin at $p < 0.05$

Table 2: MIC of compounds 1-7

Test bacteria	MIC ($\mu\text{g/mL}$)							Ampicillin ⁺
	1	2	3	4	5	6	7	
<i>Acinetobacter spp</i>	30*	≥ 30	30*	30*	30*	≥ 30	30*	-
<i>Escherichia coli</i>	30*	≥ 30	30*	30*	30*	≥ 30	30*	2 \pm 0.2
<i>Klebsiella pneumonia</i>	30*	≥ 30	30*	30*	30*	≥ 30	30*	2 \pm 0.2
<i>Pseudomonas aeruginosa</i>	15*	≥ 30	≥ 30	30*	30*	≥ 30	30*	3 \pm 0.2
<i>Staphylococcus aureus</i>	15*	≥ 30	≥ 30	30*	30*	≥ 30	30*	5 \pm 0.4
<i>Staphylococcus epidermidis</i>	15*	≥ 30	≥ 30	30*	30*	30*	30*	5 \pm 0.2
<i>Streptococcus pneumonia</i>	15*	≥ 30	30*	30*	30*	30*	30*	-

⁺Ampicillin was used as control positive, *: significant difference at $p < 0.05$

Table 3: Toxicity and antitumor activity of compounds 1-7

Test	LD ₅₀ ($\mu\text{g/mL}$)							Control ^a
	1	2	3	4	5	6	7	
Toxicity against <i>A. salina</i>	ND	ND	ND	25*	≥ 250	≥ 250	≥ 250	250
Antitumor against Erlich carcinoma cells	ND	ND	ND	50*	100	≥ 250	≥ 250	30

^aQuinidine sulfate used as positive control for toxicity assay and elspar (trade name for asparaginase) for antitumor assay, ND = not detected; *: significant difference at $p < 0.05$

Compound **2** was isolated as colorless oil. A search of chemical database using science finder found that the spectroscopic data of **2** was in accordance with the chemical structure of **nardol**, isolated previously from the rhizome of *Nardostachys jatamansi* [24]. This is the first report of ¹³C-NMR from this compound.

Compound **3** was isolated as colorless oil. The resulted spectroscopic data (*cf. exp.*) gave conclusion that the compound similar to **aristol-(9)-ene**, previously isolated from a soft coral *Lemnalia humesi* [25].

Compound **4** was isolated as colorless oil. **4** was found to be Cembrene A, a cembranoid previously isolated from the paracloacal glands of the Chinese Alligator (*Alligator sinensis*) [26].

Compound **5** was isolated as colorless oil. The spectroscopic data of **5** (*Cf. exp.*) was found to be stereochemically similar with the structure of alismoxide, a guaianediol with stereostructure 1S*, 4S*, 5S*, 10R*-4, 10-guaianediol, isolated previously from the rhizome of *Alisma plantago-aquatu* and soft coral *Sinularia sp.* [22,27].

Compound **6** was isolated as amorphous powder. The obtained spectroscopic data (*cf. exp.*) of **6** was found to be stereochemically similar with the structure of chalinasterol, a

steroid isolated previously from a soft coral of *Sinularia gibberosa* and *Lytophyton arboretum* [28,29].

Compound **7** was isolated as amorphous powder. A constructed structure based on the analysis of the spectral data (*cf. exp.*) was searched by science finder and found to be similar in structure with Nepalsterol C, a steroid isolated previously from a soft coral *Lytophyton arboreum* [29].

Biological activity assays confirmed that isoprenoids type compounds from *Lobophytum sp.* possessing antibacterial and antitumor activity. Compound **1** (alismol) has strong antibacterial activity against both gram positive (*S. aureus*, *S. epidermis*, and *S. pneumonia*) and negative bacteria (*P. aeruginosa*). Nevertheless, the antitumor activity was not detected. Compound **4** (cembrene A) showed moderate activity against all tested bacteria. Moreover, it also possessed high toxicity (25 $\mu\text{g/mL}$) against *A. salina* and appreciable antitumor activity (50 $\mu\text{g/mL}$) against Erhlich carcinoma cells.

CONCLUSION

Four sesquiterpenes, one cembranoid type diterpene and two steroids have been isolated and identified from the Red Sea soft coral

Lobophyton sp. Compound **1** (alismol) has significant antibacterial activity against gram-positive (*S. aureus*, *S. epidermis*, and *S. pneumonia*) and gram-negative bacteria (*P. aeruginosa*). Compound **4** (cembrene A) also exhibited moderate antibacterial activity against all tested bacteria and appreciable antitumor properties against Erhlich carcinoma cells. Compounds **1** and **4** display potentials for treating pathogenic bacteria associated with many diseases and/or tumor and therefore, require further investigation

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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