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Isolation and evaluation of antimicrobial and anticancer activities of brominated sesquiterpenes from Vietnamese red alga *Laurencia intermedia* Yamada

Thi Van Anh Tran[1], Van Minh Nguyen[2], Duy Hien Tran[1], Le Thanh Tuyen Nguyen[1], Thi Hong Tuoi Do[1], Thi Le Thuy Nguyen[3], Quang Ngoc Tran[2], Anh Duy Do[4], Sang Moo Kim[5,6,*]and The Han Nguyen[2,*]

[1]Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, 41 Dinh Tien Hoang Street, Ho Chi Minh City, **Vietnam**
[2]Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu Street, Nha Trang City, Khanh Hoa, **Vietnam**
[3]Biotechnology Center of Ho Chi Minh City, 2374, Highway 1, Quarter 2, Trung My Tay Ward, District 12, Ho Chi Minh City, **Vietnam**
[4]Research Department of Marine Conservation, Research Institute for Marine Fisheries, 224 Le Lai Street, Hai Phong City, **Vietnam**
[5]Department of Marine Food Science and Technology, Gangneung-Wonju National University, 7 Jukheon-gil, Gangneung 25457, **Republic of Korea**
[6]Shandong Haizhibao Marine Technology Co., Ltd., Rongcheng City, Shandong Province 264300, **China**

***Correspondence:** hannt@ntu.edu.vn **Received 09-02-2020, Revised: 09-03-2020, Accepted: 10-03-2020 e-Published: 11-03-2020**

The brominated sesquiterpenes were demonstrated to have diverse biological properties both *in vitro* and *in vivo*. In this study, three brominated sesquiterpenes including aplysistatin (**1**), palisadin A (**2**) and palisadin B (**3**) from the methanol extract of Vietnamese red alga *Laurencia intermedia* Yamada were purified and characterized. The antimicrobial activities of the purified sesquiterpenes were determined against human pathogen, shrimp and fish bacterial pathogens. The cytotoxic activities against human liver cancer (Hep-G2), breast cancer (MDA-MB-231) and muscle rhabdomyosarcoma (RD) cell lines were also evaluated. Compounds **1**-**3** exhibited the antimicrobial activities against *Vibrio harveyi* and *Edwardsiella ictalurid* with the MIC values of 250 and 500 µg/mL, respectively. All purified sesquiterpenes **1**-**3** showed the anticancer activities against Hep-G2 with the IC_{50} values of 33.98 ± 2.02, 36.70 ± 3.45 and 43.70 ± 7.18 µM, respectively. Only compound **1** exhibited the cytotoxic effects on MDA-MB-231 and RD cell lines, with the IC_{50} values of 18.86 ± 2.45 and 24.22± 2.21 µM, respectively.

Keywords: Brominated sesquiterpenes; *Laurencia intermedia* Yamada; Vietnam, anticancer activities; fishery pathogenic bacteria

INTRODUCTION

Compounds containing bromine such as bromophenols and brominated sesquiterpenes have been proved to have variety of bioactivities, such as neuroprotective, cytotoxic, antibacterial, antioxidant, antidiabetic and anti-inflammatory activities and the modulation of multidrug resistance (Liu et al., 2011; Davis and Vasanthi, 2011). Red algae are known as potential sources

of bromophenols and brominated sesquiterpenes. The genus *Laurencia* belonged to red algae is the richest source of novel brominated sesquiterpenes (Cabrita et al., 2010). Several studies have been dealt with the isolation and biological evaluation of brominated sesquiterpenes from this genus. From *Laurencia obtusa,* three brominated sesquiterpenes were isolated as (8R)-8-bromo-10-epi-β-snyderol, (8S)-8-bromo-β-snyderol, 5-bromo-3-(3‘-hydroxy-3‘-methylpent-4‘-enylidene) -2,4,4-trimethylcyclohexanone (Topcu et al., 2003). Four brominated sesquiterpenes, obtusol, (−)-elatol, dendoidiol and obtusane isolated from *Laurencia dendroidea* had anticancer activities against different cancer cell lines (U937, Jurkat, B16F10 and Colo-205) (Barcellos et al., 2018).

White spot disease is a highly contagious viral disease of the cultured shrimp caused by white spot syndrome virus (WSSV), particularly *Vibrio parahaemolyticus, Vibrio harveyi* and *Aeromonas hydrophila*. This disease causes 100% mortality in shrimps within 3-10 days of infection under farming conditions, leading to a huge economic loss to the shrimp industry (Sánchez-Paz, 2010). The lack of effective therapeutic or prophylactic measures have aggravated the situation, necessitating the development of antiviral agents. To control the disease, supplementation of antibiotics in the diets is commonly applied. Besides, several extracts from marine materials have already been tested against WSSV. For instance, the aqueous extract of brown alga *Sargassum* spp. showed significant anti-WSSV properties in marine shrimp (Immanuel et al., 2010). However, identifications of certain active compounds in algae are limited. Thus, the purification and identification of novel anti-WSSV compounds from algae are necessary to develop new drugs used for the prevention and treatment of white spot disease.

Vietnam has an abundant algal resources with nearly 1000 species. Among them, red algae are diversified and widely distributed in the Southeastern Vietnamese coast (Huynh and Nguyen, 1998). So far, the red algae are only used to prduce agar, however, studies on extracting bioactive compounds are rare. In Vietnam, the genus *Laurencia* is the most popular red alga. Fatty acid profiles of some Vietnamese *Laurencia* species have been studied (Quan et al., 2015). In the present study, we reported the isolation of three brominated sesquiterpenes from *Laurencia intermedia* Yamada harvested in Nha Trang bay, Khanh Hoa province, Vietnam and the evaluation of antimicrobial activities against shrimp and fish pathogens, and anticancer activities of the purified brominated sesquiterpenes.

MATERIALS AND METHODS

Algal samples

The red alga, *L. intermedia,* was harvested during March to July 2017 at the coast of Nha Trang bay, Khanh Hoa province, Vietnam. The samples were authenticated by Mr. Do Anh Duy (Research Institute for Marine Fisheries, Hai Phong city, Vietnam). Voucher specimens (NT-02) have been deposited at Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city. Algal samples were individually rinsed to remove particulates and any epiphytes with tap water twice, and air-dried in the shade. The dried algal samples were individually cut into small pieces (3 x 4 cm), vacuum packed in PA bags and stored at -40 °C until analysis.

2.2. General experimental procedures

1D- and 2D-NMR experiments were recorded on a Bruker Advance III 500 FT-NMR using tetramethylsilane as an internal standard, in which chloroform -*d* ($CDCl_3$) was used as solvent. High-resolution mass spectrometry (HRMS) was recorded on X500R QToF-MS (Sciex, USA), APCI-MS/ESI-MS was recorded using a single quadrupole MSQ Plus Mass Spectrometer (Thermo Fisher Scientific, USA). IR spectra were recorded on a Shimadzu IR Affinity 1-S (Tokyo, Japan). The optical rotations were determined with a P8000 A. Krüss polarimeter (Germany). Column chromatography was performed with *silica gel* (40 ∽ 63 µm, Merck) and Sephadex LH-20 (GE Healthcare Life). Thin layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Spots were detected by spraying with the vanillin – sulfuric reagent followed by heating to 105 °C.

2.3. Extraction and isolation

The dried algal sample (1.0 kg) was extracted ten times with methanol (2 L × 10 times) with ultrasound assisted extraction at room temperature. After removal of solvent under reduced pressure, the concentrated extract (0.5 L) was successively liquid–liquid extracted with *n*-hexane (0.5 L × 4 times), ethyl acetate (0.5 L × 3 times) and *n*-butanol (0.5 L × 3 times). The obtained extracts were evaporated to dryness, resulting in *n*-hexane (36.5 g), ethyl acetate (6.7 g) and *n*-butanol (4.3 g) extracts. The *n*-hexane extract was separated on *silica gel* column

chromatography using gradient solvent system of *n*-hexane–ethyl acetate (95:5 to 70:30, v/v) to obtain 31 fractions (A.1 – A.31). Fraction A.3 (0.9 g) was subjected on *silica gel* column chromatography with isocratic elution of *n*-hexane–dichloromethane (6:4, v/v) to give 6 sub-fractions (A.3.1 – A.3.6). Compound **3** (163.7 mg) was afforded from fraction A.3.3 by recrystallization in mixture of chloroform–methanol (5:5, v/v). Fraction A.9 (1.2 g) was purified by *silica gel* column chromatography, using isocratic solvent system of *n*-hexane–ethyl acetate (85:15, v/v) as mobile phase to yield compound **2** (795.2 mg). The compound **1** (1.7 g) was obtained from fraction A.18 (2.3 g) by making the recrystallization in *n*-hexane.

2.4. Characterization of isolated compounds

Aplysistatin (**1**): $C_{15}H_{21}BrO_3$, colorless crystals, α_D^{28} -30^o (*c* 0.5, $CHCl_3$) HRESIMS *m/z* 329.0719/331.0698 $[M+H]^+$; IR: 1759, 1672, 1386, 1016, 704, 590 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 0.97 (3H, s, H-13), 1.18 (3H, s, H-15), 1.30 (3H, s, H-14), 1.63 (1H, t, *J* = 3.5 Hz, H-8*α*), 1.79 (1H, td, *J* = 4.0, 13.5 Hz, H-8*β*), 2.05 (1H, dd, *J* = 2.0, 9.0 Hz, H-6), 2.12 (1H, dd, *J* = 3.5, 13.0 Hz, H-9), 2.29 (1H, dd, *J* = 3.5, 14.0 Hz, H-9), 2.55 (2H, m, H-5), 3.87 (1H, dd, *J*=7.5, 9.0 Hz, H-1α), 3.93 (1H, dd, *J* = 4.5, 13.0 Hz, H-10), 4.49 (1H, t, *J* = 9.0 Hz, H-1β), 5.13 (1H, br s, H-2), 6.95 (1H, dt, *J* = 2.5, 5.4 Hz, H-4). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C: 69.9 (t, C-1), 66.8 (d, C-2), 132.0 (s, C-3), 143.1 (d, C-4), 27.2 (t, C-5), 51.2 (d, C-6), 79.0 (s, C-7), 37.7 (t, C-8), 32.4 (t, C-9), 65.1 (d, C-10), 41.0 (s, C-11), 169.1 (s, C-12), 18.0 (q, C-13), 21.7 (q, C-14), 30.7 (q, C-15).

Palisadin A (**2**): $C_{15}H_{23}BrO_2$; colorless oil; α_D^{28} +20^o (*c* 0.5, $CHCl_3$), ESI-MS (negative): 313.35/315.25 $[M-H]^-$; IR: 1385, 1099, 700, 582 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 0.94 (3H, s, H-13), 1.17 (3H, s, H-15), 1.27 (3H, s, H-14), 1.56 (1H, dt, *J* = 3.5, 13.0 Hz, H-8β), 1.80 (1H, dt, *J* = 3.5, 13.0 Hz, H-8α), 2.08 (1H, d *J* = 9.5 Hz, H-6), 2.28 (2H, m, H-9), 2.35 (2H, m, H-5), 3.44 (1H, t, *J* = 8.5 Hz, H-1β), 3.96 (1H, dd, *J* = 4.0, 12.5 Hz, H-10), 4.07 (1H, t, *J* = 8.0 Hz, H-1α), 4.38 (2H, qd, *J* = 1.5, 13.0 Hz, H-12), 4.83 (1H, br s, H-2), 5.55 (1H, br s, H-4). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ_C: 72.7 (t, C-1), 70.1 (d, C-2), 141.9 (s, C-3), 121.7 (d, C-4), 26.3 (t, C-5), 51.8 (d, C-6), 78.0 (s, C-7), 37.6 (t, C-8), 32.7 (t, C-9), 66.3 (d, C-10), 41.0 (s, C-11), 71.1 (t, C-12), 18.0 (q, C-13), 21.9 (q, C-14), 30.8 (q, C-15).

Palisadin B (**3**): $C_{15}H_{24}Br_2O$, colorless crystals, α_D^{28} +10^o, ESI-MS (negative): 361.29/363.13/365.20 $[M-OH]^-$; IR: 1383, 1082, 1055, 667, 602 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500MHz) δ_H: 0.90 (3H, s, H-13), 1.12 (H, s, H3-15), 1.30 (3H, s, H-14), 1.65 (1H, m, H-8), 1.77 (1H, d, *J* = 10.0 Hz, H-6), 1.79 (2H, m, H-8), 2.06 (1H, dd, *J* = 8.0, 17.5 Hz, H-4), 2.13 (1H, dd, *J* = 3.5; 13.0 Hz, H-9), 2.28 (1H, dd, *J* = 4.0, 12.5 Hz), 3.37 (1H, dd, *J* = 8.5, 10.5 Hz, H-1α), 3.69 (1H, dd, *J* = 3.0, 10.5 Hz, H-1β), 4.49 (1H, m, H-2), 5.61 (1H, m, H-4), 2.13 (2H, m, H-9), 2.28 (2H, m, H-9), 3.91 (1H, dd, *J* = 4.0, 12.5 Hz, H-10); ^{13}C-NMR($CDCl_3$,125MHz) δ_C: 36.2 (t, C-1), 70.7 (d, C-2), 136.2 (s, C-3), 129.4 (d, C-4), 25.9 (t, C-5), 52.9 (d, C-6), 77.5 (s, C-7), 36.7 (t, C-8), 33.0 (t, C-9), 66.4 (d, C-10), 40.8 (s, C-11), 21.1 (q, C-12), 18.0 (q, C-13), 22.1 (q, C-14), 30.7 (q, C-15).

2.5. Determination of antimicrobial activity

The purified compounds were tested against six strains of human pathogenic bacteria; *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus MRSA* (ATCC BAA-2422), *Klebsiella pneumoniae*, *Acinetobacter baumannii* (isolated from clinical specimens) and four marine bacteria; *Vibrio parahaemolyticus, Vibrio haveyi, Aeromonas hydrophila* and *Edwardsiella ictalurid* (isolated from diseased shrimp and catfish). The antibacterial activities of purified compounds were studied using a microdilution method (Valgas et al., 2007). Bacteria were incubated in LB (Luria Bertania) culture media for 24 h at 37 °C. The bacterial suspension was diluted by LB media to 10^6 CFU/mL. The tested compounds were dissolved in DMSO (10% of the final volume) and diluted with the culture broth to get various concentrations for the assay. A 100 µL of each dilution was distributed in 96-well plates, as well as did sterility control and growth experiments (containing culture broth plus DMSO, without antimicrobial substance). For each test, growth control well was incubated with 100 µL of bacterial suspension (10^5 CFU/well). All experiments were performed in triplicate and microdilution trays were incubated at 28 °C for 24 h. Bacterial growth was detected by optical density. The minimum bactericidal concentration (MBC) values were defined as the lowest concentration of test compound which completely inhibited microbial growth. On the wells with the value of minimum bactericidal concentration (MIC), 2 MIC and 4 MIC, 100 µL of media in each well was subcultured onto the surface of the freshly prepared Mueller Hinton Agar and incubated at 37 ^{o}C for 24 h. The MBC was recorded as the lowest concentration of the

tested compounds that did not permit any visible bacteria growth after the period of incubations.

2.6. Safety assessment of aplysistatin on shrimps

The main sesquiterpene and aplysistatin of *L. intermedia* were used to assess the safety on shrimps. Asian black tiger shrimp (weighed of 3–4 g) brought from an aquaculture farms (Biotechnology Center of Ho Chi Minh city, Vietnam). Before starting the experiments, the shrimps were maintained to acclimatize in a storage tank for 2 days. The artificial seawater was adjusted to pH of 7.5-8.5, an initial salinity of 25 ppt, temperature of 26-28 °C, and DO ≥ 4 mg/L. The healthy shrimps were divided into four experimental groups (n=15 per group). One was used for the control group and the other three were used for testing at three different concentrations (250, 500 and 1000 µg/mL) of compound **1**. Compound **1** was dissolved in DMSO and diluted to different concentrations of 250, 500 and 1000 µg/mL. The mixing ratio of tested compound and pellets was 1: 4 (w/v). After impregnated with drug, the pellets were coated with cod liver oil. Shrimps were fed twice each day with pellets with or without testing compound at the amounts equivalent to 5% of shrimp body weight. The water was changed on the fourth day after feeding. The survival of shrimps was monitored for 7 days to evaluate the safety of tested compound on shrimps.

2.7. Determination of anticancer activity

Human breast cancer (MDA-MB-231) and liver cancer (HepG2) cell lines were provided by ATCC; the muscle rhabdomyosarcoma-A (RD) cell line was obtained from Pasteur Institute at Ho Chi Minh City (the origin from WHO). All the cell lines were activated and stocked in Pasteur Institute at Ho Chi Minh City. Cells were cultured in EMEM (Eagle's Minimum Essential Medium) or DMEM *(Dulbecco's Modified Eagle Medium)* supplemented with 10% FCS (fetal bovine serum), 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Tested compounds were dissolved in DMSO (10 mM) and then diluted with culture medium to get various concentrations for cell proliferation assays. Paclitaxel (Anzatax®, Mayne Pharma, New Zealand) was used as a reference compound. DMSO was used as a blank control. Cells were seeded in 96-well plates at 4.2 x10^4 cell/cm^2. After 24 h of incubation at 37 °C, 5% CO_2, 100 µL of tested compounds at the concentrations of 4, 12.5, 25, 50, 100 µM, reference compound, and blank control (DMSO) was added and incubated for 72 h at 37 °C, 5% CO_2. Cell viability was evaluated as a mitochondrial succinate dehydrogenase (SDH) activity, a marker of viable cells, using MTT test as described by (Denizot and Lang, 1986). Briefly, SDH activity was detected after 3 h incubation in culture medium without FCS containing 0.05 mg/mL MTT (3-4,5-dimethyl-thiazol-2-yl-2,5 diphenyl-tetrazolium bromide), which is converted into formazan dissolved in isopropanol by agitation for 10 min at room temperature. The produced purple solution at 570 nm was spectrophotometrically measured with Multikan™ microplate reader. The concentration to inhibit viable cells by 50% (IC_{50}) was determined from a dose-response curve, which was based on triplicate measurements.

RESULTS AND DISCUSSION

3.1. Purification and structural identification

The methanol extract of *L. intermedia* was purified by different means of chromatography that led to the purification of three compounds. The chemical structures of the purified compounds were elucidated by the analyses of their MS and NMR data, which was compared with those in the literatures. Compounds were identified as aplysistatin (**1**) (Figures S1-S5), palisadin A (**2**) (Figures S6-S10), palisadin B (**3**) (Figures S11-S15) (Su et al., 2009a).

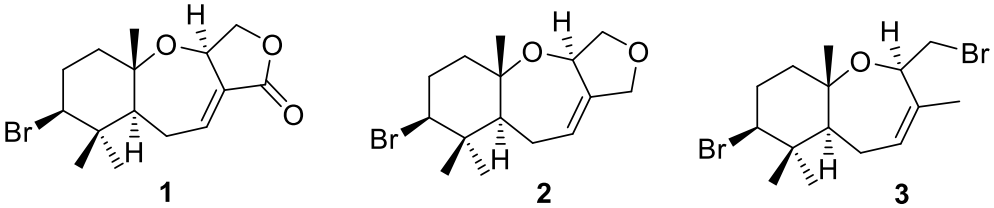

Figure 1; Structure of compounds 1–3 isolated from *Laurencia intermedia* Yamada

The structures of isolated compounds **1-3** were presented in Figure 1. Aplysistatin (**1**), palisadin A (**2**) and palisadin B (**3**), the brominated snyderane sesquiterpenes, have been identified in different species of *Laurencia* genus such as *L. saitoi* (Su et al., 2009b), *L. cf. palisada* (Paul and Fenical 1980),

L. *similis* (Su et al. 2009a) and *L. snackeyi* (Vairappan et al. 2013). Recently, aplysistatin, palisadin B were reported from *L. intermedia* collected at Ly Son island, Quang Ngai province, Vietnam (Trung et al., 2019) together with 3,4-epoxypalisadin A, 2-hydroxyluzofuranone and 2-hydroxyluzofuranone B. However, the bioactivities of these compounds were not examined. Therefore, it is first report that palisadin A was isolated from *L. intermedia* collected at Nha Trang bay, Khanh Hoa province, Vietnam. Moreover, the bioactivities (anticancer and antibacterial properties) of these compounds were firstly evaluated. Interestingly, concentrations of purified compounds from *L. intermedia* harvested from Khanh Hoa province (aplysistatin up to 0.17% in dried sample) were much higher compared to those harvested from Quang Ngai province (0.015% in dried sample). The differences in brominated components and their concentrations could be due to the variations in environmental conditions (e.g., temperature and salinity).

3.2. Antimicrobial activity

The purified sesquiterpenes **1**-**3** were tested for their antimicrobial activities against six human pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus aureus*, *MRSA Klebsiella pneumonia* and *Acinetobacter baumannii*) and four shrimp and fish pathogen bacteria (*Vibrio parahaemolyticus, Vibrio haveyi, Aeromonas hydrophila* and *Edwardsiella ictalurid)*. The results indicated that aplysistatin, palisadin A and palisadin B had less or no effects on human pathogen (MIC values >500 µg/mL) (data not shown), in which antimicrobial activities were determined against the WSPV *Vibrio harveyi* (MIC value of 250 µg/mL) and *Edwardsiella ictalurid* caused enteric septicaemia of catfish (MIC value of 500 µg/mL) (Table 1).

Table 1; Antimicrobial activities of compounds (1-3) against shrimp and fish bacterial pathogens

Compounds	Organism							
	V. parahaemolyticus		***V. harveyi***		***A. hydrophila***		***E. ictaluri***	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
Palisadin A	>500	-	250	250	>500	-	500	500
Palisadin B	>500	-	250	250	>500	-	500	500
Aplysistatin	>500	-	250	250	>500	-	250	250

The MBC/MIC ratio used to identify whether the active compound was a bactericidal or a bacteriostatic compound (Kone et al., 2004). Since the results of MBC/MIC ratio of three brominated sesquiterpenes are equal to one. Thus, aplysistatin, palisadin A and B could be considered to be the bactericidal agents. In order to use these isolated compounds to treat shrimp and fish diseases, it is necessary to investigate their safety. Therefore, aplysistatin, a main sesquiterpene in *L. intermedia,* was assessed the safety on shrimp. The survival rates of black tiger shrimps fed with aplysistatin at all tested concentrations (250, 500 and 1000 µg/mL) remained 100% during 7 days of experiment (data not shown). These results indicated that aplysistatin isolated from *L. intermedia* was safe on shrimps and could be used as a veterinary medicine.

The algal brominated sesquiterpenes have been demonstrated to have different biological activities. However, limited information on antimicrobial activity of these compounds has been reported. So far, there has been only one publication on the antimicrobial activities against fish and human pathogens of two chamigrene-type sesquiterpenes purified from *L. chondrioides* (Bansemir et al., 2004). In this study, aplysistatin, palisadin A and B were found as the antibiotics against *Vibrio harveyi* and *Edwardsiella ictalurid*. *Vibrio harveyi,* a marine Gram-negative luminous organism, is a serious pathogen of marine fish and invertebrates, particularly penaeid shrimps (Austin and Zhang, 2006). *Edwardsiella ictalurid,* a Gram-negative bacterium, causes enteric septicemia of catfish which is a problem in the catfish industry (Williams et al. 2012). Previously, two chamigrene-type sesquiterpenes purified from CH_2Cl_2 extract of *L. chondrioides* showed weak inhibition of the growth of microorganisms (Bansemir et al., 2004). In this study, aplysistatin, palisadin A and B were found to be active. The differences in antibacterial activities may be due to the differences in structure of compounds. Due to the resistance of antibiotics for food animals, there is a need for new antibacterial compounds, which are safe and limit the risk of transfer of antibiotic resistance to humans. The findings of sesquiterpenes from *L. intermedia* were active

against shrimp and fish pathogenic bacteria, and safe in aquaculture for shrimps. This could open a new application for algal compounds in aquaculture as a natural veterinary medicine.

3.3. Anticancer activity

The anticancer activities of sesquiterpenes **1**, **2** and **3** were assessed against three human cancer cell lines (HepG2, MDA-MB-231 and RD) using MTT assay (Table 2). All tested compounds showed antiproliferative effect against the liver cancer cells (HepG2) with IC_{50} of 33.9, 36.7 and 43.7 µM, respectively. Compound **1** (aplysistatin) exhibited the cytotoxicity activity in the breast cancer cells (MDA-MB-231) and the muscle rhabdomyosarcoma cells (RD) with IC_{50} of 18.86 and 24.22 µM, respectively (Figure 2, Table 2), while compounds **2** and **3** had weak activity (IC_{50} >100 µM). In comparison with the activity of paclitaxel, a popular anticancer drug, used in clinical practice to treat ovarian, breast, and other carcinomas, the anticancer activities of these isolated compounds were slightly lower (Table 2).

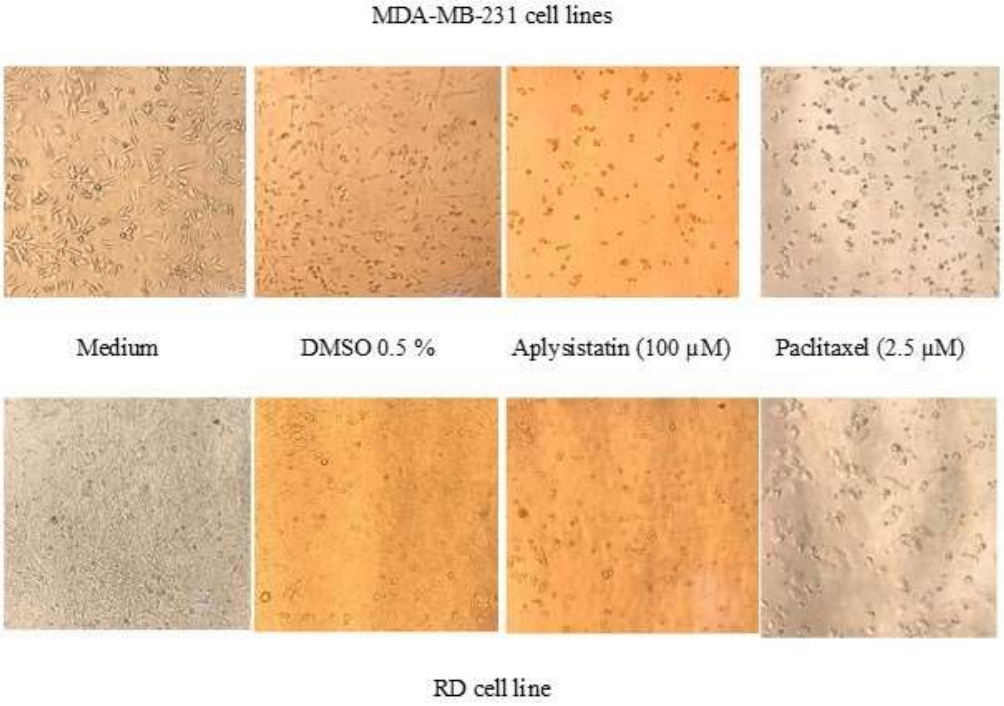

Figure 2; Morphological changes in Aplysistatin-treated MDA-MB 231 and RD cells

Table 2; Anti-proliferation activity of compounds (1-3) on cell lines (mean ± SD, n=3)

Compounds	IC_{50} (µM)		
	HepG2	MDA-MB-231	RD
1	33.98 ± 2.02	18.86 ± 2.45	24.22 ± 2.21
2	36.70 ± 3.45	>100	>100
3	43.70 ± 7.18	>100	>100
Paclitaxel	11.96 ± 4.46	11.06 ± 3.19	7.59 ± 2.61

Previously, aplysistatin was interested due to significant inhibitory activity against murine lymphocytic leukemia P-388 in the screening of National Cancer Institute (Pettit et al., 1997). This compound together with palisadin A and palisadin B exhibited the cytotoxic properties against KB cells (König and Wright, 1993), but were found to be inactive in cytotoxicity assay against the tumor cell line BEL7402 (Su et al., 2009a). In the report on the biological activities of selected marine natural products, aplysistatin was found as a potential anticancer agent with the activity against ten cell lines (BC1, HT, Lu1, MeI2, CoI2, KB, KB-V1, P-388, LNCaP, ZR-75-1) with EC_{50} ranging from 1.2 to 17.0 µg/mL, while palisadin A and B had limit activity on some cell lines (MeI2, KB, KB-V1, P-388, LNCaP, ZR-75-1) (König et al., 1994). The assessment cytotoxic activity of aplysistatin, palisadin A and B on three cell lines

(HepG2, RD and MDA-MB-231) in this study provided more information on the anticancer activities of red algal metabolites. Additionally, the difference in the structures of aplysistatin, palisadin A and B and the potential effects in cell lines suggest that the question about role of lactone ring connected with snyderane skeleton. Further studies need to be carried out to find out the anticancer mechanisms of these compounds.

CONCLUSION

This study reported antibacterial and anticancer activities of three brominated sesquiterpenes (aplysistatin, palisadin A and palisadin B) puried from Vietnamese *L*. *intermedia*. The purified compounds showed inhibitory activities against bacterial shrimp and fish pathogens (*Vibrio harveyi* and *Edwardsiella ictalurid*) and cancer cell lines (Hep-G2, MDA-MB-231 and RD). The present study revealed that *Laurencia* brominated sesquiterpenes could be a potential source as antimicrobial and anticancer agents. Therefore, these algal compounds could be used to prevent and treat white spot disease of shrimp and enteric septicemia of catfish. These compounds could also be a good candidate for the development of human anticancer drugs.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

T.V.A. Tran, T.H. Nguyen, V.M. Nguyen and S.M. Kim designed both isolation and bioactivity experiments. A.D. Do contributed to seaweed sample collection, identification and treatment. T.V.A. Tran, T.H. Nguyen, D.H. Tran, L.T.T. Nguyen, T.H.T. Do and Q.N. Tran performed the isolation experiments. T.H. Nguyen, T.L.T. Nguyen and V.M. Nguyen carried out the evaluation of bioactivities. T.H. Nguyen, T.V.A. Tran, V.M. Nguyen and S.M. Kim wrote and finalized the manuscript.

REFERENCES

Austin, B., & Zhang, X. H. (2006). *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. *Letters in Applied Microbiology*, *43*(2), 119-124.

Bansemir, A., Just, N., Michalik, M., Lindequist, U., & Lalk, M. (2004). Extracts and sesquiterpene derivatives from the red alga *Laurencia chondrioides* with antibacterial activity against fish and human pathogenic bacteria. *Chemistry & Biodiversity*, *1*(3), 463-467.

Barcellos, M. M., Rodrigues de Freitas, W., Lacerda da Silva Machado, F., Correa Ramos Leal, I., Ribeiro Soares, A., Masahiko Kanashiro, M., & Frazão Muzitano, M. (2018). Cytotoxic activity of halogenated sesquiterpenes from *Laurencia dendroidea*. *Phytotherapy Research*, *32*(6), 1119-1125.

Cabrita, M. T., Vale, C., & Rauter, A. P. (2010). Halogenated compounds from marine algae. *Marine Drugs*, *8*(8), 2301-2317.

Davis, G. D. J., & Vasanthi, A. H. R. (2011). Seaweed metabolite database (SWMD): A database of natural compounds from marine algae. *Bioinformation*, *5*(8), 361.

Denizot, F., & Lang, R. (1986). Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods*, *89*(2), 271-277.

Huynh N. Q. & Nguyen, H. D, “The seaweed resources of Vietnam. In: Alan TC, Masao O (eds.) Seaweed resources of the world”. Yokosuka, Japan: Japan International Cooperation Agency, pp. 62–69, 1998.

Immanuel, G., Sivagnanavelmurugan, M., Balasubramanian, V., & Palavesam, A. (2010). Effect of hot water extracts of brown seaweeds *Sargassum* spp. on growth and resistance to white spot syndrome virus in shrimp Penaeus monodon

postlarvae. *Aquaculture Research*, *41*(10), 545-553.

Kone, W. M., Atindehou, K. K., Terreaux, C., Hostettmann, K., Traore, D., & Dosso, M. (2004). Traditional medicine in North Côte-d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, *93*(1), 43-49.

König, G. M., & Wright, A. D. (1993). Algal secondary metabolites and their pharmaceutical potential. *American Chemical Society*, 534, 276-293.

König, G. M., Wright, A. D., Sticher, O., Angerhofer, C. K., & Pezzuto, J. M. (1994). Biological activities of selected marine natural products. *Planta Medica*, *60*(6), 532-537.

Liu, M., Hansen, P. E., & Lin, X. (2011). Bromophenols in marine algae and their bioactivities. *Marine Drugs*, *9*(7), 1273-1292.

Paul, V. J., & Fenical, W. (1980). Palisadins A, B and related monocyclofarnesol-derived sesquiterpenoids from the red marine alga *Laurencia cf. palisada*. *Tetrahedron Letters*, *21*(29), 2787-2790.

Pettit, G. R., Herald, C. L., Allen, M. S., Von Dreele, R. B., Vanell, L. D., Kao, J. P., & Blake, W. (1977). Antineoplastic agents. 48. The isolation and structure of aplysistatin. *Journal of the American Chemical Society*, *99*(1), 262-263.

Quan, P. M., Anh, N. V. T., Tien, D. D., Hue, P. T., & Long, P. Q. (2015). Study on using data of fatty acids in botanical classification (chemotaxonomy) for red seaweed species. *Vietnam Journal of Marine Science and Technology*, *15*(3), 273-279.

Sánchez-Paz, A. (2010). White spot syndrome virus: an overview on an emergent concern. *Veterinary Research*, *41*(6), 43.

Su, H., Shi, D. Y., Li, J., Guo, S. J., Li, L. L., Yuan, Z. H., & Zhu, X. B. (2009a). Sesquiterpenes from *Laurencia similis*. *Molecules*, *14*(5), 1889-1897.

Su, H., Yuan, Z. H., Li, J., Guo, S. J., Deng, L. P., Han, L. J., Zhu, X. B, & Shi, D. Y. (2009b). Sesquiterpenes from the marine red alga *Laurencia saitoi*. *Helvetica Chimica Acta*, *92*(7), 1291-1297.

Topcu, G., Aydogmus, Z., Imre, S., Gören, A. C., Pezzuto, J. M., Clement, J. A., & Kingston, D. G. (2003). Brominated sesquiterpenes from the red alga *Laurencia obtusa*. *Journal of Natural Products*, *66*(11), 1505-1508.

Trung, D. V., Truc, N. T. T., Duy, C. N. H., Nhan, L. T. T., Phung, N. K. P., & Duong, N. T. T. (2019). Halogenated sesquiterpenes from the red alga *Laurencia intermedia* Yamada. *Vietnam Journal of Chemistry*, *57*(6), 723-727.

Vairappan, C. S., Kamada, T., Lee, W. W., & Jeon, Y. J. (2013). Anti-inflammatory activity of halogenated secondary metabolites of *Laurencia snackeyi* (Weber-van Bosse) Masuda in LPS-stimulated RAW 264.7 macrophages. *Journal of Applied Phycology*, *25*(6), 1805-1813.

Valgas, C., Souza, S. M. D., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 369-380.

Williams, M. L., Gillaspy, A. F., Dyer, D. W., Thune, R. L., Waldbieser, G. C., Schuster, S. C., ... & Lawrence, M. L. (2012). Genome sequence of *Edwardsiella ictaluri* 93-146, a strain associated with a natural channel catfish outbreak of enteric septicemia of catfish. *Journal of Bacteriology*, *194*(3), 740-741.