(1→6)-β-D-glucopyranoside (**8**). The structures of these compounds were elucidated by NMR spectroscopic analysis and comparison with the reported data. Among these, phenolic glycosides are the predominant compounds. Compounds **1-8** showed weak or fewer effects against the tested cancer cell lines. This is the first time that glycoside constituents and their cytotoxic activity were reported from *M. sinensis*, which was established based on the extensive literature search in the SciFinder database. Further studies should focus on the diversity of metabolites of the genus *Miliusa*.

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CYCLOPEPTIDES FROM THE MARINE ACTINOMYCETE *DIETZIA* SP. G727 DERIVED FROM THE SEAWEED *LOBOPHORA* SP.

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Summary

Cyclopeptides from the Marine Actinomycete *Dietzia* sp. G727 Derived from the Seaweed *Lobophora* sp.

From the methanol extract of the *Dietzia* sp. G727 derived from the seaweed *Lobophora* sp., seven compounds were isolated including staphylopeptide A (**1**), cyclo-(L-Pro-L-Asn) (**2**), cyclo-(L-Pro-L-Ser) (**3**), cyclo-(L-Pro-L-Thr) (**4**), cyclo-(L-Ala-4-hydroxy-L-Pro) (**5**), adenine (**6**) and adenosine (**7**). Their structures were elucidated by the nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and comparison with the reference data. Compounds **3**, **5** and **6** showed good antimicrobial activity against *Enterococcus faecalis* and *Candida albicans* with the MIC values in the range from 32 to 128 µg/mL. In addition, only compound **6** inhibited Gram-negative bacteria *Escherichia coli* with a MIC value of 64 µg/mL. To the best of our knowledge, this is the first report of the isolation of staphylopeptide A from *Dietzia* genus.

Keywords: *Dietzia genus, Marine actinomycete, Cyclodipeptide, Cyclotetrapeptide, Antimicrobial activity.*

1. Introduction

Marine actinobacteria have been known as an important source of natural products which possess bioactivities such as anticancer, antioxidant, antifungal, antiviral, etc [1]. Among them, *Dietzia* is a small genus in the marine actinomycete family and this genus has been considered as a potential source of enzymes for the usage of the biodegradation, bioremediation, industrial fermentation, and carotenoid pigmentation [2].

In the search for new bioactive compounds from marine microorganisms in Vietnam, the methanol extract of the *Dietzia* sp. (strain G727) isolated from the seaweed *Lobophora* sp. collected in the Ninh Thuan coast province exhibited antimicrobial activity against three Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579) and one yeast strain *Candida albicans* ATCC 10231 with MIC values of 32, 16, 32, and 8 µg/mL, respectively. In this paper, we report the isolation and structural elucidation of seven compounds from the culture broth of the seaweed-derived actinomycete *Dietzia* sp. (strain G727) (Fig. 1). The antimicrobial activity of the isolated compounds was also discussed.

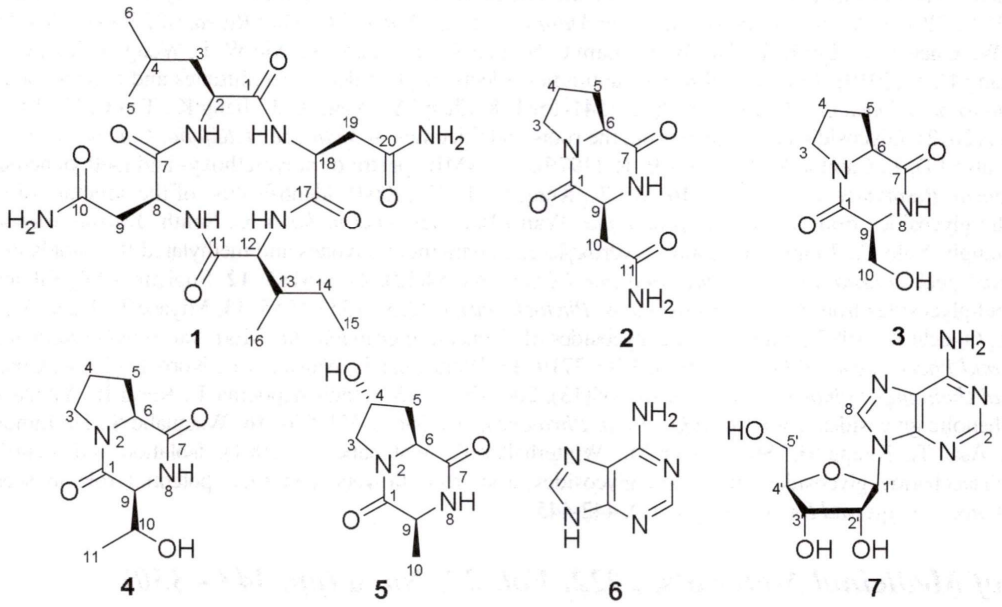

Fig. 1. Structures of isolated compounds **1-7** from *Dietzia* sp. G727

2. Material and methods

2.1. Marine materials

Strain G727 was isolated from the seaweed *Lobophora* sp. collected at a depth of 8 meters, from the coast of Ninh Thuan province in April 2021 and was identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources - Vietnam Academy of Science and Technology (VAST). A voucher specimen (NT-R016) was deposited at the Institute of Marine Environment and Resources, VAST.

2.2. General experimental procedures

ESI-MS spectra were recorded on an Agilent 1100 LC-MSD Trap spectrometer. NMR spectra were recorded on a Bruker 600 MHz spectrometer. Solvents for NMR spectra were $CDCl_3$, CD_3OD, and DMSO-d_6 using TMS as an internal standard. TLC *silica gel* Merck 60 F_{254} was used for thin-layer chromatography (TLC) and preparative TLC. Column chromatography (CC) was carried out using *silica gel* (230-400 mesh, Merck), *silica gel* RP-18 (30-50 µm, YMC) or Sephadex LH-20 (Sigma). Fractions were monitored by TLC. Spots were visualized by UV light (254 nm and 365 nm) and by heating *silica gel* plates sprayed with sulfuric acid 10% reagent. Medium pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.3. Isolation, identification, and fermentation of the actinomycete G727 strain

The seaweed sample (0.5 g) was crushed by a

glass chopstick in a falcon tube; 4.5 mL of sterile sea water was added, then the mixture was homogenized by vortexing for 1 minute, and the suspension was treated using a wet-heat technique (60°C for 6 minutes). After that, 0.5 mL suspension was transferred to 4.5 mL sterile distilled water and vortexed for 1 minute afforded the sample solution. Aliquots of 50 µL of sample solution were spread on M1 medium pH 7.0 (g/L: 1.0 g soluble starch, 0.4 g yeast extract, 0.2 g peptone, 30.0 g instant ocean salt, 15.0 g agar) supplemented with 50 µg/mL polymycin B and cycloheximide to inhibit Gram-negative bacterial and fungal contaminations. After 7 days of aerobic incubation at 30°C, the colony of the G727 actinomycete strain was transferred onto a petri dish of A1 medium (g/L: 5.0 g soluble starch, 2.0 g yeast extract, 1.0 g peptone, 30.0 g instant ocean salt, 15.0 g agar) for purification (Fig. 2) and this strain was identified belonging to the *Dietzia* genus by using 16S rRNA gene sequence analysis.

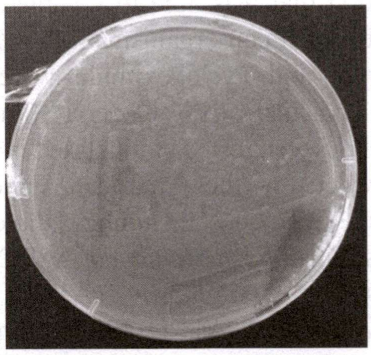

Fig. 2. Morphological appearance of G727 strain's colonies

Strain G727 was activated and inoculated into a flask containing 2.5 L of A1 broth medium. After 7 days of incubation at 30°C with the agitation of 150 rpm, the culture broth was used as seed culture to inoculate into 50 L of high-nutrient medium $A1^+$ (soluble starch: 10.0 g/L, yeast extract: 4.0 g/L, peptone: 2.0 g/L, instant ocean salt: 30.0 g/L, KBr (20.0 mg/mL): 5.0 mL, $FeSO_4$ (8.0 mg/mL): 5.0 mL, $CaCO_3$: 1.0 g/L). The fermentation was incubated at 30°C with the agitation of 150 rpm and harvested on the twelve days.

2.4. Isolation of chemical constituents from the methanol extract of Dietzia sp. G727

The culture broth (50 L) of G727 strain was filtered and absorbed over the Amberlite column, then eluted with MeOH solvent. The solution was concentrated *in vacuo*, to give 188.7 g of the MeOH extract. The MeOH extract was separated by chromatography on a Diaion column, using solvent mixtures of H_2O/MeOH gradient (0-100% MeOH) as the eluents to yield 8 fractions F1-F8. Fraction F5 (4.1 g) was subjected to a Sephadex LH-20 column, eluted with MeOH/H_2O (8/2) to give 4 sub-fractions F5.1-F5.4. Sub-fraction F5.3 (0.21 g) was further purified by gel filtration over *silica gel* column chromatography, eluted with CH_2Cl_2/MeOH gradient to give 10 sub-fractions. Sub-fraction F5.3.3 (40.2 mg) was subjected to a *silica gel* column, eluted with *n*-hexane/acetone gradient to give **5** (4.6 mg). Sub-fraction F5.3.4 (20 mg) was separated on a Sephadex LH-20 column, eluting with MeOH, and followed by recrystallization in acetone/MeOH (6/4) to give **4** (6.2 mg). Sub-fraction F5.3.5 (15 mg) and F3.3.7 (10 mg) were crystallized with CH_2Cl_2/MeOH (4/6) to give **3** (3.8 mg) and **2** (1.3 mg), respectively. Sub-fraction F5.4 (0.39 g) was further purified by gel filtration over Sephadex LH-20 column chromatography with MeOH as eluent to give **6** (4.0 mg). Fraction F6 (2.9 g) was separated on a Sephadex LH-20 column, eluting with a mixture of MeOH/H_2O (8/2) to give 4 sub-fractions F6.1-F6.4. Sub-fraction F6.3 (0.74 g) was chromatographed by reversed phase column chromatography on a medium pressure liquid chromatography system (MPLC), eluted with a gradient solvent mixture of MeOH/H_2O to yield 4 sub-fractions F6.1-F6.4. Sub-fraction F6.3 (78.3 mg) was subjected to a *silica gel* column chromatography, eluted with CH_2Cl_2/MeOH gradient to give **7** (1.9 mg). Fraction F8 (2.5 g) was further purified by gel filtration over Sephadex LH-20 column chromatography with MeOH as eluent to give 4 sub-fractions F8.1-F8.4. Sub-fraction F8.2 (1.2 g) was subjected to a *silica gel* column chromatography, eluted with CH_2Cl_2/MeOH gradient to give 10 sub-fractions F8.2.1-F8.2.10. Sub-fraction F8.2.5 (30 mg) was separated on a *silica gel* column chromatography using a mixture of CH_2Cl_2/MeOH (9/1) to yield **1** (1.8 mg).

Staphylopeptide A (1): White solid, $[\alpha]_D^{25}$ = -50.2 (*c* 0.015, MeOH) (**ref 4:** $[\alpha]_D^{30}$ = -57.8 (c 0.0575, pyridine)). HRESI-MS: *m/z* 455.2609 $[M+H]^+$, ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6): see Table 1.

Cyclo-(L-Pro-L-Asn) (2): White solid, $[\alpha]_D^{25}$ = -70.5 (*c* 0.02, MeOH), ^{1}H-NMR (600MHz, DMSO-d_6), δ_H (ppm): 1.77 (1H, m, H_a-5), 1.79 (1H, m, H_a-4), 1.86 (1H, m, H_b-4), 2.15 (1H, m, H_b-5), 2.43 (1H, dd, J = 4.2, 15.0 Hz, H_a-10), 2.54 (1H, dd, J = 7.8, 15.0 Hz, H_b-10), 3.33 (1H, overlapped, H_a-3), 3.43 (1H, m, H_b-3), 3.96 (1H, m, H-9), 4.12 (1H, m, H-6), 6.96 (1H, br. s, NH_2), 7.42 (1H, br. s, NH_2), 8.08 (1H, s, NH-8). ^{13}C-NMR (150 MHz, DMSO-d_6), δ_C (ppm): 21.6 (C-4), 28.4 (C-5), 38.5 (C-10), 44.9 (C-3), 54.1 (C-9), 57.6 (C-6), 165.1 (C-1), 168.4 (C-7), 170.7 (C-11).

Cyclo-(L-Pro-L-Ser) (3): White solid, $[\alpha]_D^{25}$ = -34.7 (*c* 0.0525, MeOH), ^{1}H-NMR (600MHz, CD_3OD), δ_H (ppm): 1.84 (1H, m, H_a-4), 1.91 (1H, m, H_a-5), 2.06 (1H, m, H_b-4), 2.35 (1H, m, H_b-5), 3.35 (1H, m, H_a-3), 3.63 (1H, m, H_b-3), 3.74 (1H, dd, J = 3.0, 11.4 Hz, H_a-10), 3.89 (1H, t, J = 2.4 Hz, H-9), 3.97 (1H, dd, J = 3.0, 11.4 Hz, H_b-10), 4.32 (1H, dd, J = 6.6, 10.8 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 22.8 (C-4), 30.1 (C-5), 46.5 (C-3), 60.2 (C-10), 61.3 (C-9), 65.3 (C-6), 167.2 (C-1), 172.5 (C-7).

Cyclo-(L-Pro-L-Thr) (4): White solid, $[\alpha]_D^{25}$ = -95.5 (*c* 0.025, MeOH), ^{1}H-NMR (600MHz, CD_3OD), δ_H (ppm): 1.26 (3H, d, J = 6,6 Hz, CH_3-11), 1.81-1.96 (2H, m, H_a-4, H_a-5), 2.06 (1H, m, H_b-4), 2.35 (1H, m, H_b-5), 3.53 (1H, m, H_a-3), 3.61 (1H, m, H_b-3), 3.69 (1H, m, H-9), 4.21 (1H, m, H-10), 4.32 (1H, dd, J = 6.6, 10.2 Hz, H-6). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 20.0 (C-11), 22.8 (C-4), 30.0 (C-5), 46.5 (C-3), 60.1 (C-10), 64.6 (C-9), 70.5 (C-6), 166.7 (C-1), 172.5 (C-7).

Cyclo-(L-Ala-4-hydroxy-L-Pro) (5): White solid, $[\alpha]_D^{25}$ = -16.1 (*c* 0.0038, MeOH), ESI-MS: *m/z* 185.5 $[M+H]^+$. ^{1}H NMR (600 MHz , CD_3OD), δ_H (ppm): 1.41 (3H, d, J = 6.6 Hz, H-10), 2.11 (1H, m, H_a-5), 2.31 (1H, m, H_b-5), 3.45 (1H, d, J = 12.6 Hz, H_a-3), 3.70 (1H, dd, J = 4.2, 12.6, Hz, H_b-3), 4.26 (1H, q, J = 6.6 Hz, H-9), 4.49 (1H, t, J = 4.2 Hz, H-6), 4.54 (1H, m, H-4), ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 15.2 (C-10), 36.9 (C-5), 51.5 (C-9), 54.5 (C-3), 58.2 (C-6), 68.6 (C-4), 169.4 (C-7), 173.0 (C-1).

Adenine (6): White solid, ESI-MS: *m/z* 136 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 8.13 (1H, s, H-8), 8.20 (1H, s, H-2).

Adenosine (7): White solid; ESI-MS (*m/z*): 268.1 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm) 3.77 (1H, dd, J = 3.0, 12.6 Hz, H_a-5), 3.91 (1H, dd, J = 2.4, 12.6 Hz, H- H_b-5), 4.19 (1H, dd, J = 2.4, 5.4 Hz, H-4), 4.35 (1H, dd, J = 3.0, 4.8 Hz, H-3), 4.76 (1H, dd, J = 5.4, 6.6 Hz, H-2), 5.99 (1H, d, J = 6.6 Hz, H-1), 8.20 (1H, s, H-8), 8.32 (1H, s, H-2).

2.5. Evaluating antimicrobial activity of the G727 strain

Antimicrobial activity test using the serial dilution method of Hadecek (2000) [3] was carried out at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology. The samples were diluted in DMSO with the decrease in concentration from 256 µg/mL, 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL and 2 µg/mL. Next, 50 µL of bacteria and yeast solution at a concentration of 2.10^5 CFU/mL were added and the mixture was incubated at 37°C for 24 hours. The MIC value was determined at the sample with the lowest concentration that was able to completely inhibit the growth of microorganisms after 24 hours. Streptomycin and cycloheximide were used as positive controls for bacteria and yeast, respectively. Seven tested strains used in this study were provided by the American Type Culture Collection (ATCC), including three Gram-negative strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076; three Gram-positive strains: *Enterococcus faecalis* ATCC 29212, *Staphylococus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579 and one yeast strain *Candida albicans* ATCC 10231. The independent experiments were performed in triplicate.

3. Results and discussion

Compound **1** was obtained as white amorphous powder and was optically active $[\alpha]_D^{25}$ = -50.2 (*c* 0.015, MeOH). Its molecular formula was determined as $C_{20}H_{34}N_6O_6$ based on the proton adduct ion $[M+H]^+$ at *m/z* 455.2609 $[M+H]^+$ (calcd. for $C_{20}H_{35}N_6O_6$, *m/z* 455.2618) in the HR-ESI mass spectrum, together with the ^{13}C-NMR data. Thus, seven degrees of unsaturation were deduced for **1**. The ^{1}H-NMR spectrum of **1** showed the presence of three doublet methyls at δ_H 0.92 (J = 7.2 Hz, H_3-16),

0.88 (J = 6.6 Hz, H_3-5), 0.86 (J = 6.6 Hz, H_3-6), one triplet methyl at δ_H 0.84 (J = 7.2 Hz, H_3-15), and other aliphatic protons at δ_H 1.41-4.20 ppm. In addition, four broad singlet protons the down field at δ_H 8.12 (1H, s, NH-2), 8.02 (1H, s, NH-12), 7.83 (1H, s, NH-18), 7.73 (1H, s, NH-8) were also noted. Analysis of the ^{13}C-NMR spectrum with the aid of an HSQC experiment revealed the resonances of twenty carbons including four methyl groups at δ_C 23.0 (CH_3-5), 21.8 (CH_3-6), 15.0 (CH_3-16), 11.9 (CH_3-15), four methylene groups at δ_C 42.2 (C-3), 38.6 (C-9), 38.1 (C-19), 24.2 (C-14), six methine groups at δ_C 58.6 (C-12), 52.5 (C-2), 51.3 (C-18), 50.1 (C-8), 37.7 (C-13), 23.6 (C-4), and six carbonyl carbons at δ_C 166.9 (C-11), 167.7 (C-17), 167.8 (C-7), 168.5 (C-1), 171.3 (C-20), 171.1 (C-10). The chemical shifts of C-2, C-8, C-12, and C-18 suggested their linkages to nitrogen. These data together with the unsaturation degree of **1** suggested that **1** was a cyclic tetrapeptide.

In the ^{1}H-^{1}H COSY spectrum of **1**, the presence of four spin-spin interaction systems: NH-2/H-2/H-3/H-4/H-5, H-6, NH-8/H-8/H-9, NH-18/H-18/H-19, NH-12/H-12/H-13/H-14/H-15, H-16 which could be assigned to four amino acid residues: isoleucine (Ile), leucine (Leu), and two asparagines (Asn) were observed. The sequence Ile-Asn1-Leu-Asn2 and cyclic form of **1** were confirmed by the HMBC correlations of H-2 (δ_H 3.78) with C-7 (δ_C 167.8), C-1 (δ_C 168.5), H-8 (δ_H 4.20) with C-7 (δ_C 167.8), C-10 (δ_C 171.1), H-12 (δ_H 3.77) with C-17 (δ_C 167.7), C-11 (δ_C 166.9), and H-18 (δ_H 4.14) with C-17 (δ_C 167.7),C-20 (δ_C 171.3) (Fig. 3). Moreover, the correlations between H-8/2-NH (δ_H 4.14/8.12), H-2/18-NH (δ_H 3.78/7.83), H-18/12-NH (δ_H 4.20/8.02), and H-12/8-NH (δ_H 3.77/7.73) in the NOESY spectrum which were in accordance with L-configurations of natural amino acids established the structure of **1**. Careful analyses of the 2D-NMR spectra of **1** and comparison with the reference data determined the structure of **1** as staphylopeptide A [4]. To the best of our knowledge, this is the first report of the isolation of staphylopeptide A from *Dietzia* genus.

Table 1. NMR data of compound **1**

Position	δ_H mult. (J in Hz)	δ_C (type)	HMBC interaction (H-C)
Leu			
1	-	168.5 (C)	-
2	3.78 m	52.5 (CH)	C-1, C-7
3	1.54 m 1.62 m	42.2 (CH_2)	C-2, C-4, C-6
4	1.83 m	23.6 (CH)	C-3, C-5
5	0.88 d (6.6)	23.0 (CH_3)	C3, C-4, C-6
6	0.86 d (6.6)	21.8 (CH_3)	C-3, C-4, C-5
2-NH	8.12 s	-	C-8
Asn^1			
7	-	167.8 (C)	
8	4.14 t (4.8)	50.1 (CH)	C-7, C-10
9	2.31 dd (7.8, 15.6) 2.68 dd (5.4, 15.6)	38.6 (CH_2)	C-7, C-8, C-10
10	-	171.1 (C)	-
8-NH	7.73 s		C-12
Ile			
11	-	166.9 (C)	-
12	3.77 m	58.6 (CH)	C-11, C-13, C-14
13	1.86 m	37.7 (CH)	
14	1.19 m 1.41 m	24.2 (CH_2)	C-13, C-15, C-16
15	0.84 t (7.2)	11.9 (CH_3)	C-13, C-14
16	0.92 d (7.2)	15.0 (CH_3)	C-12, C-13, C-14
12-NH	8.02 s		
Asn^2			
17	-	167.7 (C)	-

Position	δ_H mult. (*J* in Hz)	δ_C (type)	HMBC interaction (H-C)
18	4.20 t (4.8)	51.3 (CH)	C-17, C-19, C-20
19	2.40 dd (7.2, 15.6) 2.61 dd (4.8, 15.6)	38.1(CH_2)	C-17, C-20
20	-	171.3 (C)	
18-NH	7.83 s	-	
NH_2-10	6.95 br. s 7.43 br. s		C-9
NH_2-20	7.41 br. s 6.93 br. s		C-19

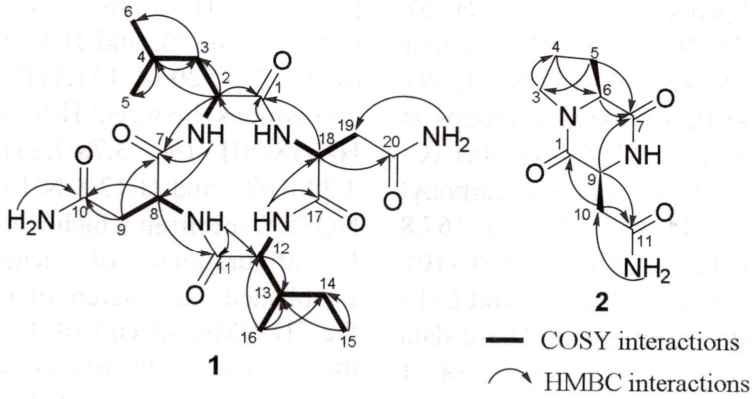

Fig. 3. Selected ^{1}H-^{1}H COSY and HMBC correlations of **1** and HMBC correlations of **2**

Compound **2** was isolated as a white solid. Analysis of the ^{13}C-NMR spectra with the aid of the HSQC experiment revealed the resonances of nine carbons including four methylene groups at δ_C 21.6 (C-4), 28.4 (C-5), 38.5 (C-10), 44.9 (C-3), two methine groups at δ_C 54.1 (C-9), 57.6 (C-6) and three carbonyl groups at δ_C 165.1 (C-1), 168.4 (C-7), 170.7 (C-11)]. The chemical shifts of C-3, C-6, and C-9 suggested their linkages to oxygen or nitrogen. The ^{1}H-NMR spectrum with the support of the HSQC spectrum of **2** indicated the presence of two methines bearing nitrogen at δ_H 3.96 (1H, m, H-9), 4.12 (1H, m, H-6), four methylene groups including one methylene bearing nitrogen at δ_H 3.33 (1H, overlapped, H_a-3), 3.43 (1H, m, H_b-3) and the remaining methylenes at δ_H 1.77 (1H, m, H_a-5), 2.15 (1H, m, H_b-5), 1.79 (1H, m, H_a-4), 1.86 (1H, m, H_b-4), 2.43 (1H, dd, J = 4.0, 15.0 Hz, H_a-10), 2.54 (1H, dd, J = 7.8, 15.0 Hz, H_b-10), along with the signals of three amine groups at δ_H 8.08 (1H, s, NH-8), δ_H 6.96 (1H, s, NH_2), and 7.42 (1H, s, NH_2). In the HMBC spectrum of **2**, the correlations of H-5 with C-3, C-7, H-4 with C-6, C-5, C-3, H-3 with C-4, C-5, C-6, H-6 with C-7, C-5 determined the positions of C-3, C-4, C-5, C-6. The positions of C-9, C-10, C-11 were located by the correlations of H-9 to C-11, C-7, C-1, H-10 to C-11, C-1, C-9, NH-8 to C-6 and C-9, NH_2 to C-10 (Fig. 3). Detailed analysis of the 1D and 2D-NMR spectra data of **2** and comparison with the literature data indicated that compound **2** was cyclo-(L-Pro-L-Asn) [5].

Compound **3** was isolated as a white solid. In the ^{1}H-NMR and ^{13}C-NMR spectra of **3**, it was worth noting that the NMR signals of **3** were close to those of **2**, except for the disappearance of an amide group in **3**. The chemical shifts of proton H-10 at δ_H 3.74 (1H, dd, J = 3.0, 11.4 Hz, H_a-10), 3.97 (1H, dd, J = 3.0, 11.4 Hz, H_b-10) and C-10 at δ_C 60.2 indicated that C-10 was linked with an oxygen atom. Thus, a complete analysis of the 1D-NMR of **3** and comparison with the reference data determined compound **3** as cyclo-(L-Pro-L-Ser) [5],[6].

Compound **4** was isolated as a white solid. Analysis of the ^{13}C-NMR spectrum of **4** showed the presence of nine carbons, including one methyl at δ_C 20.0 (C-11), three methylene groups at δ_C 22.8 (C-4), 30.0 (C-5), 46.5 (C-3), three methine groups at δ_C 60.1 (C-10), 64.6 (C-9), 70.5 (C-6) and two carbonyl groups at δ_C 166.7 (C-1), 172.5 (C-7). The chemical shifts of C-3, C-6, C-9, and C-10 suggested their linkage to

oxygen or nitrogen. The ^{1}H-NMR spectrum of **4** showed the presence of one methyl group at δ_H 1.26 (3H, d, J = 6,6 Hz, CH_3-11), methine and methylene groups bearing nitrogen or oxygen at δ_H [3.69 (1H, d, J = 2.4 Hz), 4.21 (1H, m), 4.32 (1H, dd, J = 6.6, 10.2 Hz), 3.53 (1H, m), 3.61 (1H, m)], and other aliphatic protons at δ_H 1.81-2.35. Complete analysis of the ^{1}H-NMR, ^{13}C-NMR of **4**, and comparison with the reported values indicated that compound **4** was cyclo-(L-Pro-L-Thr) [5],[6].

Compound **5** was isolated as a white solid. The ESI-MS spectrum of **5** showed the pseudomolecular ion peak at *m/z* 185.5 $[M+H]^+$. The ^{1}H-NMR spectrum of **5** indicated the presence of one methyl group at δ_H 1.41 (3H, d, J = 6.6 Hz, H-10), three methine groups bearing nitrogen or oxygen at δ_H 4.26 (1H, q, J = 6.6 Hz, H-9), 4.49 (1H, t, J = 4.2 Hz, H-6), 4.54 (1H, m, H-4), two methylene groups including one nitrogen-bearing methylene at δ_H 3.45 (1H, d, J = 12.6 Hz, H_a-3), 3.70 (1H, dd, J = 4.2, 12.6 Hz, H_b-3) and another methylene at δ_H 2.11 (1H, m, H_a-5), 2.31 (1H, m, H_b-5). Analysis of the ^{13}C-NMR spectra of **5** showed the presence of eight carbons, including one methyl at δ_C 15.2 (C-10), three methine groups at δ_C 51.5 (C-9), 58.2 (C-6), 68.6 (C-4), two methylene groups at δ_C 36.9 (C-5), 54.5 (C-3), two carbonyl groups at δ_C 169.4 (C-7), 173.0 (C-1). These data were characteristics of a dipeptide compound. Complete analysis of the 1D-NMR, mass spectrum and comparison with the reference data indicated that compound **5** was cyclo-(L-Ala-4-hydroxy-L-Pro) [7].

The structures of **6-7** were identified based on their MS and NMR data and comparison with the literature data as adenine (**6**) [8] and adenosine (**7**) [9].

Among the isolated compounds, compounds **1**, **3-6** were evaluated for their antibacterial activity against *Enterococcus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923) *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13076), and anti-yeast property against *Candida albicans* (ATCC 10231) (Table 2). Unfortunately, compound **1** was inactive against all of the tested microorganism. Compounds **3**, **5** and **6** showed good antimicrobial activity against *E. faecalis* and *C. albicans* with MIC values in the range of 32 to 128 µg/mL. Most of the compounds were inactive against Gram-negative bacteria, except compound **6**. This compound inhibited Gram-negative bacteria *Escherichia coli* (ATCC 25922) with a MIC value of 64 µg/mL.

Table 2. Antimicrobial activity of compounds **1**, **3 - 6**

Compound	Gram (+)			Gram (-)			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
	MIC (µg/mL)						
1	-	-	-	-	-	-	-
3	64	-	-	-	-	-	128
4	128	-	-	-	-	-	-
5	64	-	64	-	-	-	128
6	32	-	-	64	-	-	64
Streptomycin	256	256	128	32	256	128	
Cycloheximide							32

Positive controls: Streptomycin; Cycloheximide.

4. Conclusion

Analysis of an antimicrobial extract prepared from culture broth of the marine-derived actinomycete *Dietzia* sp. G727 led to the isolation of seven compounds identified as staphylopeptide A (**1**), cyclo-(L-Pro-L-Asn) (**2**), cyclo-(L-Pro-L-Ser) (**3**), cyclo-(L-Pro-L-Thr) (**4**), Cyclo-(L-Ala-4-hydroxy-L-Pro) (**5**), adenine (**6**) and adenosine (**7**). Compounds **3**, **5** and **6** showed good antimicrobial activity against *E. faecalis* and *C. albicans* with MIC values in the range of 32 to 128 µg/mL. Most of compounds were inactive against Gram negative bacteria, except for compound **6**. This compound inhibited Gram-negative bacteria *Escherichia coli* (ATCC 25922) with a MIC value of 64 µg/mL.

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POLYPRENYLATED ACYLPHLOROGLUCINOLS FROM THE FRUIT PERICARP OF *GARCINIA OBLONGIFOLIA* AND THEIR CYTOTOXICITY

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Summary

Polyprenylated Acylphloroglucinols from the Fruit Pericarp of *Garcinia oblongifolia* and their Cytotoxicity

The paper deals with chromatographic isolation, structural elucidation, and cytotoxic activity of compounds from the pericarp of *Garcinia oblongifolia* fruit. Four compounds, isogarcinol, oblongifolin A, garcinol, and 1,5-dimethyl citrate were isolated. Standard spectroscopic methods (MS, 1D and 2D NMR) were used in the evaluation of the structures of the four compounds. The SRB cytotoxicity test showed micromolar half-maximal inhibitory concentrations (IC_{50}) of isogarcinol against Hep-G2, HeLa, and Vero cell lines and garcinol against Hep-G2, HeLa, LU-1, and Vero cell lines.

Keywords: *Garcinia oblongifolia, Acylphloroglucinol, Citrate, Cytotoxicity.*

1. Introduction

The genus *Garcinia* covers nearly 260 species and is one of the largest genera of the Clusiaceae family. The highest abundance of this species is distributed in Southeast Asia [1]. A vast number of studies have been conducted on *Garcinia* phytochemistry with about 135 species investigated. Xanthones, benzophenones, biflavonoids, and triterpenoids have been isolated and the connection of their structures with antidiabetic and cytotoxic activities was disclosed in the studies of *Garcinia* species which find the habitat in Vietnam [2],[3],[4],[5]. *Garcinia oblongifolia* Champ. ex Benth. is a common edible plant in Vietnam. The fruits have been harvested from the wild or cultivated plant. Polyprenyl benzoylphloroglucinols and xanthones were isolated from different parts (bark, twig, leaves, trunk, and fruit) of *G. oblongifolia* [4],[6],[7],[8],[9]. Hydroxycitric acid (HCA) is the main constituent of *G. oblongifolia* fruit. Except for a few reports on the