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SECONDARY METABOLITES FROM THE MARINE-DERIVED FUNGUS *ASPERGILLUS VERSICOLOR* M852

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Summary

Secondary Metabolites from the Marine-Derived Fungus *Aspergillus versicolor* M852

Ten compounds were isolated from the culture of *Aspergillus versicolor* M852, an endophytic fungus isolated from the marine mollusca at Hon Cau 2, Binh Thuan, Vietnam. These compounds include (2*R*)-2,3-dihydro-7-hydroxy-6,8-dimethyl-2-[(*E*)-prop-1-enyl]-chroman-4-one (**1**), fumigaclavine I (**2**), fumigaclavine C (**3**), cyclo-(Pro-Phe) (**4**), cyclo-(Pro-Tyr) (**5**), cyclo-(Pro-Ala) (**6**), cyclo-(Pro-Val) (**7**), norharman (**8**), phloretic acid (**9**), and 3-phenylpropanoic acid **(10)**. Their structures were identified through MS and NMR data analyses. These compounds were then assessed for their antimicrobial activity against a range of clinically significant microorganisms. Most of them exhibited inhibitory activity against one to three tested Gram-positive strains, with MIC values ranging from 64 to 256 µg/mL.

Keywords: *Aspergillus versicolor, Antimicrobial activity, Dipeptide.*

1. Introduction

Marine fungi present a valuable source of secondary metabolites with significant potential for pharmaceutical development. These metabolites encompass a diverse array of compounds, such as terpenes, steroids, polyketides, peptides, alkaloids, and polysaccharides. These compounds play a crucial role in conferring antimicrobial, anticancer, antiviral, antioxidant, and anti-inflammatory activities on marine fungi [1],[2]. Ever since Alexander Fleming's groundbreaking discovery of penicillin in 1928 from *Penicillium* [3], the search for novel drugs derived from fungi has never ceased [4].

Vietnam stands out as one of the world's leaders in marine biodiversity. During our screening program for antimicrobial compounds from some selected microorganisms in the sea of Central Vietnam, we discovered that the extract from the *Aspergillus versicolor* M852 strain displayed significant antimicrobial activity against various microorganisms. Specifically, it exhibited an antibacterial effect against two Gram-positive bacteria strains, *Enterococcus faecalis* ATCC29212 and *Bacillus cereus* ATCC14579, as well as the Gram-negative bacteria strain *Salmonella enterica* ATCC13076 and the yeast *Candida albicans* ATCC10231 strain, with MIC values of 64, 32, 128 and 256 µg/ml, respectively. In this study, we present the isolation of ten compounds from the marine fungus *Aspergillus versicolor* M852 strain (Fig. 1), which were isolated from mollusca samples collected at Hon Cau 2 in Binh Thuan, Vietnam. Additionally, we discuss the antimicrobial activity exhibited by these isolated compounds.

2. Materials and Methods

2.1. Marine materials

Strain M852 was isolated from the Mollusca, collected at a depth of 8 meters, from the seacoast of Binh 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 (BT-204e) was deposited at the Institute of Marine Environment and Resources, VAST.

2.2. General experiment procedures

Melting points were recorded on a Boetius apparatus (Germany). ESI-MS was obtained using an Agilent 1100 instrument (United States). 1D and 2D NMR spectra were recorded on Bruker Avance 500 MHz and 600 MHz spectrometers (Germany). 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 a 10%

sulfuric acid reagent. Medium-pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.3. Isolation, identification, and fermentation of the fungus M852 strain

The Mollusca 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 to afford the sample solution. Aliquots of 50 µL of sample solution were spread on ISP1 medium pH 7.0 (g/L: 5.0 g cation, 3.0 g yeast extract, 30.0 g instant ocean salt) supplemented with 50 µg/mL polymycin B and cycloheximide to inhibit Gram-negative bacterial and fungal contamination. After 7 days of aerobic incubation at 30°C, the colony of the M852 fungus 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. 1) and this strain was identified as *Aspergillus versicolor* by using 16S rRNA gene sequence analysis.

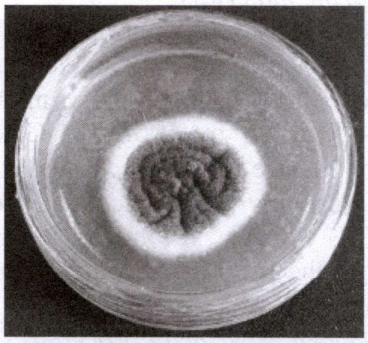

Fig. 1. Morphological appearance of M852 strain's colonies

Strain M852 was activated and inoculated into a 2.5 L of A1 broth medium flask. 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 an agitation rate of 150 rpm and harvested after twelve days.

2.4. Extraction and isolation

The culture broth (50 L) of the *Aspergillus versicolor* M852 strain was filtrated, absorbed over Amberlite XAD 16, and eluted with methanol (50 L) to give the solution. The solution was concentrated *in vacuo* to give 181.2 g of MeOH residue. The residue was suspended in water (1.5 L) and partitioned with EtOAc (4 × 2 L each) to obtain EtOAC fraction (EA, 85 g) and a water phase. The EA fraction (85 g) was chromatographed on a *silica gel* chromatography column (CC), eluting with a solvent gradient of *n*-hexane/acetone (10→100 % acetone, v/v) to yield 8 fractions, F1-F8. Fraction F5 (3.3 g) was separated on a *silica gel* CC with a mixture of CH_2Cl_2/MeOH gradient (0→50% MeOH, v/v) as eluents to give 6 fractions, F5.1-F5.6. Fraction F5.3 (0.35 g) was continuously separated on a Sephadex LH-20 CC and eluted with MeOH to obtain five fractions F5.3.1 - F5.3.5. Fraction 5.3.3 (45 mg) was crystallized in CH_2Cl_2/MeOH (9/1) to yield compound **9** (12 mg). Fraction F6 (3.8 g) was separated on a Sephadex LH-20 CC using MeOH/H_2O (9/1, v/v) as eluent to give 5 fractions, F6.1- F6.5. Fraction F6.3 (0.25 g) was further purified by gel filtration over Sephadex LH-20 CC eluting with MeOH to give 4 fractions, F6.3.1- F6.3.4. Fraction F6.3.2 (48 mg) was crystallized in CH_2Cl_2/MeOH (9/1, v/v) to yield compound **10** (8 mg). Fraction F6.4 (0.7 g) was continuously separated on a *silica gel* CC and eluted with a CH_2Cl_2/MeOH gradient to yield compound **6** (10 mg). Chromatography of fraction F7 (4.6 g) over Sephadex LH-20 CC using MeOH as eluent gave 7 fractions, F7.1 - F7.7. Purification of F7.1 (2.2 g) by a *silica gel* CC with a mixture of CH_2Cl_2/MeOH gradient as eluent yielded compound **7** (5 mg). Fraction F7.4 (0.42 g) was continuously separated on a silica gel CC, eluted with CH_2Cl_2/acetone gradient to give compounds **8** (8 mg) and **4** (4 mg). Fraction F8 (5.4 g) was further purified by gel filtration over Sephadex LH-20 CC, eluted with MeOH to give 6 fractions F8.1- F8.6. Fraction F8.3 (0,35 g) was subjected to *silica gel* CC, eluted with a CH_2Cl_2/acetone gradient to give compounds **1** (5 mg) and **5** (6 mg). Fraction F8.4 (0.68 g) was separated on a silica gel CC using CH_2Cl_2/ acetone gradient as eluents to give compounds **2** (10 mg) and **3** (7 mg).

(2*R*)-2,3-Dihydro-7-hydroxy-6,8-dimethyl-2-[(*E*)-prop-1-enyl]-chroman-4-one (**1**): White solid, mp 184-185°C; $[\alpha]_D^{25}$ −2.1° (*c* 0.03, CH_3OH); HR-ESI-MS *m/z* 255.0990 $[M + Na]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.57 (1H, s, H-5), 5.88 (1H, dq, *J* = 15.5, 6.5 Hz, H-10),

5.71 (1H, dq, J = 15.5, 6.0 Hz, H-9), 5.28 (1H, s, OH), 4.85 (1H, m, H-2), 2.69 (2H, m, H-3), 2.20 (3H, s, H-12), 2.14 (3H, s, H-13), 1.78 (3H, d, J = 6.5 Hz, H-11). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 8.0 (C-13), 15.3 (C-12), 17.8 (C-11), 42.7 (C-3), 78.2 (C-2), 110.6 (C-8), 114.5 (C-4′),117.1 (C-6), 125.8 (C-5), 128.9 (C-9), 129.7 (C-10), 158.7 (C-7), 159.4 (C-8′), 191.7 (C=O).

Fumigaclavine I (2): White powder, $[\alpha]_D^{25}$ −73.4° (c 0.05, CH_3OH), ESI-MS m/z 399 $[M+H]^+$, ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 12.26 (1H, s, NH), 8.68 (1H, d, J = 8.5 Hz, H-14), 7.49 (1H, t, J = 8.0 Hz, H-13), 6.93 (1H, d, J = 8.0 Hz, H-12), 6.13 (1H, dd, J = 10.5, 17.5 Hz, H-22), 5.54 (1H, t, J = 2.5 Hz, H-9), 5.32 (1H, d, J = 10.5 Hz, H_a-23), 5.37 (1H, d, J = 17.5 Hz, H_b-23), 3.22 (1H, t, J = 10.0 Hz, H-10), 3.20 (1H, d, J = 16.0 Hz, H-3a), 2.66 (1H, m, H-5), 2.55 (1H, d, J = 16.0 Hz, H-3b), 2.56-2.64 (2H, m, H-7), 2.32 (3H, s, H-17), 2.11 (1H, m, H-8), 1.90 (3H, s, H-25), 1.42 (6H, s, H-20, H-21), 1.30 (3H, d, J = 7.5 Hz, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 16.5 (C-18), 21.0 (C-25), 24.7 (C-20+ C-21), 33.1 (C-8), 41.8 (C-10), 42.4 (C-17), 45.0 (C-3), 46.9 (C-19), 56.5 (C-7), 59.1 (C-5), 71.0 (C-9), 114.7 (C-23), 118.8 (C-14), 118.9 (C-16), 119.3 (C-12), 135.6 (C-13), 142.4 (C-15), 142.5 (C-22), 143.3 (C-11), 170.5 (C-24), 176.3 (C-2), 200.9 (C-4).

Fumigaclavine C (3): White solid, $[\alpha]_D^{25}$ −85.8° (c 0.05, CH_3OH), ESI-MS: m/z 367 $[M+H]^+$, ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 7.15 (1H, d, J = 8.5 Hz, H-14), 7.00 (1H, t, J = 7.5 Hz, H-13), 6.67 (1H, d, J = 7.0 Hz, H-12), 6.17 (1H, dd, J = 10.5, 17.0 Hz, H-22), 5.70 (1H, br. s, H-9), 5.12 (1H, d, J = 17.0 Hz, H-23a), 5.08 (1H, d, J = 10.5 Hz, H-23b), 3.70 (1H, dd, J = 5.0, 14.5 Hz, H-4a), 3.58 (1H, m, H-10), 3.32 (1H, m, H-5), 3.29 (1H, m, H-7a), 3.18 (1H, dd, J = 4.0, 12.5 Hz, H-7b), 2.91 (1H, dd, J = 11.5, 14.5 Hz, H-4b), 2.85 (3H, s, H-17), 2.29 (1H, m, H-8), 1.86 (3H, s, H-25), 1.56 (3H, s, H-20), 1.55 (3H, s, H-21), 1.37 (3H, d, J = 8.0 Hz, H-18). ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm) 16.0 (C-18), 20.8 (C-25), 27.2 (C-4), 28.0 (C-21), 28.1 (C-20), 33.7 (C-8), 39.3 (C-10), 40.2 (C-19), 43.2 (C-17), 58.2 (C-7), 63.5 (C-5), 71.1 (C-9), 104.1 (C-3), 109.7 (C-14), 111.8 (C-23), 113.20 (C-12), 122.7 (C-13), 127.3 (C-11), 128.4 (C-16), 134.4 (C-15), 139.3 (C-2), 147.3 (C-22), 172.1 (C-24).

Cyclo-(Pro-Phe) (4): White solid, $[\alpha]_D^{25}$ -77.2 (c 0.04, MeOH). ESI-MS: m/z 245 $[M+H]^+$, ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm): 5.77 (1H, br. s, NH), 4.28 (1H, dd, J = 3.0, 9.5, H-9), 4.07 (1H, t, J = 7.5 Hz, H-6), 3,62 (1H, m, H_b-3), 3.54 (1H, m, H_a-3), 2.80 (1H, dd, J = 10.0, 14.5 Hz, H_a-10), 2.35 (1H, m, H_b-10), 2.03 (2H, m, H_b-4, H_b-5), 1.92 (2H, m, H_a-4, H_a-5). ^{13}C-NMR (100 MHz, CD_3OD): δ_C (ppm) 21.1 (C-4), 28.4 (C-5), 39.6 (C-10), 44.7 (C-3), 57.7 (C-9), 58.4 (C-6), 127.1 (C-4′), 128.3 (C-3′, C-5′), 129.9 (C-2′, C-6′), 135.3 (C-1′), 166.0 (C=O), 170.0 (C=O).

Cyclo-(Pro-Tyr) (5): White solid, $[\alpha]_D^{25}$ -80.5 (c 0.05, MeOH). ESI-MS: m/z 261 $[M+H]^+$, ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 7.06 (2H, d, J = 8.5 Hz, H-3′, H-5′), 6.73 (2H, d, J = 8.5 Hz, H-2′, H-6′), 4.87 (OH-phenol), 4.36 (1H, m, H-9), 4.05 (1H, m, H-6), 3.80 (2H, d, J = 5.0 Hz, H-10), 3.63 (1H, t, J = 6.0 Hz, H_b-3), 3.55 (1H, m, H_a-3), 2.71 (1H, t, J = 5.5 Hz, H_b-5), 2.16 (1H, m, H_b-4), 1.80 (2H, m, H_a-4, H_a-5).

Cyclo-(Pro-Ala) (6): White solid, mp. 140-141°C, $[\alpha]_D^{25}$ -78.5 (c 0.05, MeOH). ^{1}H-NMR (600 MHz, CD_3OD): $\ddot{a}_H$ (ppm) 4.27 (1H, m, H-9), 4.20 (1H, m, H-6), 3.54 (2H, m, H-3), 2.32 (1H, m, H_b-5), 2.05 (2H, m, H_b-4, H_a-5), 1.95 (1H, m, H_a-4), 1.40 (3H, d, J = 7.2 Hz, H-10). ^{13}C-NMR (125 MHz, CD_3OD): $\ddot{a}_C$ (ppm) 15.7 (CH_3-10), 23.6 (C-4), 29.2 (C-5), 46.4 (C-3), 52.1 (C-9), 60.5 (C-6), 169.1 (C-1), 172.6 (C-7).

Cyclo-(Pro-Val) (7): White solid, $[\alpha]_D^{25}$ -88.5 (c 0.04, MeOH). ESI-MS: m/z 197 $[M+H]^+$, ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 4.07 (1H, t, J = 7.0 Hz, H-6), 3.93 (1H, br. s, H-9), 3.54 (2H, m, H-3), 2.64 (1H, m, H-10), 2.38 (1H, m, H_b-5), 2.05 (1H, m, H_a-5), 2.03 (1H, m, H_b-4), 1.92 (1H, m, H_a-4), 1.05 (3H, d, J = 6.5 Hz, H-12), 0.92 (3H, d, J = 6.5 Hz, H-11). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 15.2 (C-11), 17.3 (C-12), 21.8 (C-4), 28.1 (C-5), 28.5 (C-10), 44.8 (C-3), 58.6 (C-9), 60.1 (C-6), 166.2 (C=O), 171.1 (C=O).

Norhaman (8): White solid, ^{1}H-NMR (600 MHz, $CDCl_3$): 8.92 (1H, s, H-2), 8.50 (1H, br. s, NH), 8.47 (1H, d, J= 5.4 Hz, H-1), 8.14 (1H, d, J = 7.8 Hz, H-8), 7.96 (1H, d, J = 4.8 Hz, H-11), 7.57 (1H, td, J = 1.2, 7.8 Hz, H-5), 7.55 (1H, t, J = 8.4 Hz, H-6), 7.30 (1H, td, J = 1.2, 7.2 Hz, H-7).

Phloretic acid (9): White solid, ESI-MS: m/z 167 $[M+H]^+$, ^{1}H-NMR (600 MHz, CD_3OD): $\ddot{a}_H$ (ppm) 7.11 (2H, d, J = 7.8 Hz, H-2, H-6), 6.79 (2H, d, J = 7.8 Hz, H-3, H-5), 3.14 (2H, t, J = 8.4 Hz, H-7), 2.90 (2H, t, J = 8.4 Hz, H-8).

3-Phenylpropanoic acid (10): White solid, ^{1}H-NMR (600 MHz, CD_3OD): $\ddot{a}_H$ (ppm) 7.27 (2H, d, J = 7.2 Hz), 7.24 (2H, t, J = 7.2 Hz), 7.16 (1H, t, J = 7.2 Hz), 2.92 (1H, t, J = 8.4 Hz, H-1′), 2.56 (1H, t, J = 8.4 Hz, H-2′).

2.5. Evaluation of antimicrobial activity

Antimicrobial activity test using the serial dilution method of Hadecek (2000) [5] was carried out at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology. The samples were diluted in DMSO in the decreasing concentration range 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 for the sample with the lowest concentration that was able to completely inhibit the growth of microorganisms after 24 hours. Streptomycin and cycloheximide antibiotics were positive controls for bacteria and yeast, respectively. Seven tested strains were used in this study, which were provided by the American Type Culture Collection (ATCC), including three Gram-negative strains (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076), three Gram-positive strains (*Enterococcus faecalis* ATCC29212, *Stapphylococus aureus* ATCC25923 and *Bacillus cereus* ATCC 14579) and one yeast strain (*Candida albicans* ATCC10231). The independent experiments were performed in triplicate.

3. Results and discussion

Compound **1** was isolated as a white solid, $[\alpha]_D^{25}$ = -2.1° (*c* 0.03, MeOH), and its molecular formula of $C_{14}H_{16}O_3$ was established by the sodium adduct peak $[M+Na]^+$ at *m/z* 255.0990 (calculated for $C_{14}H_{16}O_3Na$, *m/z* 255.0997) in the HR-ESI-MS. Analysis of the ^1H NMR spectrum of **1** revealed the signals of three methyl groups at δ_H 2.14 (3H, s), 2.20 (3H, s), and 1.78 (3H, d, J = 6.5 Hz), one aromatic proton at δ_H 7.57 (1H, s), two olefinic protons at δ_H 5.88 (1H, dq, J = 15.5, 6.5 Hz), 5.71 (1H, dd, J = 15.5, 6.0 Hz). In addition, the signals of three aliphatic protons were also observed at δ_H 2.69 (2H, m) and 4.85 (1H, m) in the ^1H-NMR spectrum. The ^{13}C NMR and DEPT spectra with the aid of HSQC spectrum of **1** showed the presence of fourteen carbons, including one carbonyl group (δ_C 191.7), three methyl groups (δ_C 8.0, 15.3, and 17.8), one methylene group (δ_C 42.7), one oxymethine group at δ_C 78.2 (C-2), three sp^2-methine groups [δ_C 117.1 (C-6), 128.9 (C-9) and 129.7 (C-10)], and five non-protonated carbons. In the COSY spectrum of **1**, the spin-spin interaction system of protons H-3 (δ_H 2.69)/H-2 (δ_H 4.85)/H-9 (δ_H 5.71)/H-10 (δ_H 5.88)/H-11 (δ_H 1.78) was observed. The above spectral evidence indicated that compound **1** could be a derivative of 4-chromanone containing two methyl groups and one propenyl group. In the HMBC spectrum of **1**, correlations from H-3 (δ_H 2.69) to C-2 (δ_C 78.2), C-9 (δ_C 128.9) and C-4 (δ_C 191.7), from H-9 (δ_H 5.71) to C-2, C-10 and C-11, from H-10 (δ_H 5.88) to C-2, C-9 and C-11, and from H-11 to C-9 and C-10 proved the location of the propenyl group at C-2. The HMBC correlations of proton at δ_H 2.20 (CH_3-12) with C-5, C-6, and C-7 along with correlations of H-5 (δ_H 7.57) with C-4, C-6, and C-7 allowed the determination of the methyl group (CH_3-12) at C-6. Similarly, the methyl group (CH_3-13) was determined at C-8 based on the HMBC correlations of H-13 with C-8, C-7, and C-8'. The *trans*-configuration of the C-9/C-10 double bond was determined by a large coupling constant of H-9 to H-10 (J = 15.5 Hz). Based on the careful analysis of the HR-MS, 2D-NMR spectra and comparison with reference data, compound **1** was determined as (2*R*)-2,3-dihydro-7-hydroxy-6,8-dimethyl-2-[(*E*)-prop-1-enyl]-chroman-4-one [6]. This compound has been reported to have cytotoxic activity against the cancer cell line MCF-7 with an IC_{50} value of 9.51 µg/mL [7],[8].

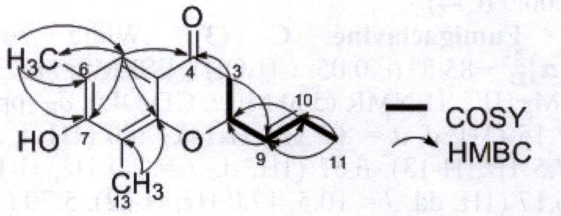

Fig. 2. Selected ^1H-^1H COSY and HMBC correlations of compound **1**

Compound **2** was obtained as a white solid. Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 399. The ^1H NMR spectrum of **2** showed the signals of five methyl groups at $\ddot{a}_H$ 2.32 (3H, s, H-17), 1.90 (3H, s, H-25), 1.42 (6H, s, H-20 and H-21), 1.30 (3H, d, J = 7.5 Hz, H-18), three aromatic protons at $\ddot{a}_H$ 8.68 (d, J = 8.5 Hz, H-14), 7.49 (t, J = 8.0 Hz, H-13), 6.93 (d, J = 8.0 Hz, H-12) and three olefinic protons at $\ddot{a}_H$ 6.13 (1H, dd, J = 10.5, 17.5 Hz, H-22), 5.32 (1H, d, J = 10.5 Hz, H_a-23), 5.37 (1H, d, J = 17.5 Hz, H_b-23). Additionally, the signals of aliphatic protons were also observed at $\ddot{a}_H$ 2.11- 3.30 ppm. The singlet signal at δ_H 12.26 (1H, s) was assigned to an NH group by analysis of the

HSQC spectrum. The ^{13}C NMR and DEPT spectra with the aid of the HSQC experiment revealed the resonances of twenty-three carbons, including five methyl groups, three methylene groups [with one sp^2methylene group at $\ddot{a}_C$ 114.7 (C-23)], eight methine groups [with four sp^2methine groups at $\ddot{a}_C$ 135.6 (C-13), 119.3 (C-12), 118.8 (C-14) and 142.5 (C-22)]; four quaternary carbons at $\ddot{a}_C$ 46.9 (C-19), 142.4 (C-15), 118.9 (C-16) and 143.3 (C-11), one ketone group at $\ddot{a}_C$ 200.9 (C-4) and two carbonyl groups of ester or amide at $\ddot{a}_C$ 176.3 (C-2) and 170.5 (C-24). In the ^1H-^1H COSY spectrum of **2**, four spin-spin interaction systems, including H-5 ($\ddot{a}_H$ 2.66)/H-10 ($\ddot{a}_H$ 3.20)/H-9 ($\ddot{a}_H$ 5.54)/H-8 ($\ddot{a}_H$ 2.11)/H-7, H-12 ($\ddot{a}_H$ 6.93)/H-13 ($\ddot{a}_H$ 7.49)/H-14 ($\ddot{a}_H$ 8.68), H-8 ($\ddot{a}_H$ 2.11)/H-18 ($\ddot{a}_H$ 1.30), and H-22 ($\ddot{a}_H$ 6.13)/H-23 ($\ddot{a}_H$ 5.33) were indicated the presence of four fragments shown in Fig. 3.

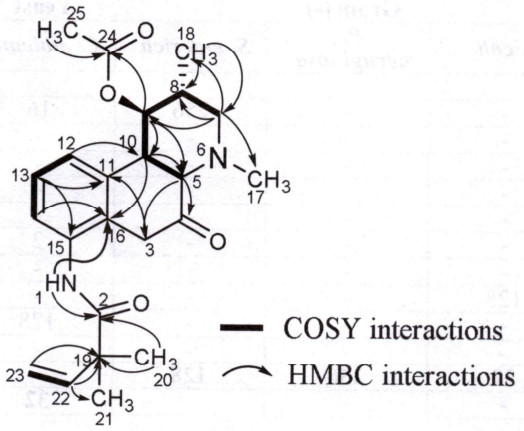

Fig. 3. Selected ^1H-^1H COSY and HMBC correlations of **2**

The HMBC correlations from H-7 to C-5, C-8, C-9, C-17 and C-18, from H-9 to C-5, C-7, C10 and C-24, from H-25 to C-24, from H-18 to C-7, C-8 and C-9, from H-17 to C-5 and C-7 indicated the existence of a six-membered ring (C5-N6-C7-C8-C9-C10) with an acetoxy group at C-9. The HMBC correlations from H-5 to C-3, C-4, C-10, and C-11, and from H-14 to C-16 confirmed the existence of a tricyclic structure. The correlations from H-23 to C-19 and C-22, from H-22 to C-2, C-19, C-20, and C-21, and from H-20/H-21 to C-19, C-22, and C-2, along with ^1H-^1H COSY correlation between H-23 and H-22 allowed the identification of an isopentenyl group at C-2. The HMBC correlation of proton in the NH group with C-2 and C-15 together with the above spectral evidence suggested that the structure of compound **2** could be in accordance with fumigaclavine I [9]. In the NOESY spectrum, the NOE cross-peaks between H-10 and H-9, H_3-18, but not with H-5 suggested that H-9, H-10, and CH_3-18 were oriented on the same side and assigned as α-positions. In addition, a medium NOE correlation was observed between H_3-25 and H-5 indicating β-orientation of H-5. Based on the careful analysis of the HR-MS, 2D-NMR spectra, and comparison with reference data [9],[10], compound **2** was determined as fumigaclavine I.

Compound **3** was obtained as a white solid, $[\alpha]_D^{25}$ −85.8° (*c* 0.05, CH_3OH). Its positive ESI-MS showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 367. The ^1H NMR spectrum exhibited the signals of five methyl groups at $\ddot{a}_H$ 2.85 (3H, s), 1.86 (3H, s), 1.55 (3H, s), 1.56 (3H, s) and 1.37 (3H, d, J = 8.0 Hz), three protons of a 1,2,3 tri-substituted benzene ring at $\ddot{a}_H$ 7.15 (d, J = 8.5 Hz, H-14), 7.00 (t, J = 7,5 Hz, H-13) and 6.67 (d, J = 7.0 Hz, H-12); and three olefinic protons at $\ddot{a}_H$ 6.17 (1H, dd, J = 10.5, 17.0 Hz, H-22), 5.12 (1H, d, J = 17.0 Hz, H-23a) and 5.08 (1H, d, J = 10.5 Hz, H-23b). Besides, the aliphatic protons were resonated at $\ddot{a}_H$ 2.29-3.70 ppm. The ^{13}C NMR and DEPT spectra with the aid of the HSQC experiment revealed the resonances of twenty-three carbons, including five methyl groups, three methylene groups, eight methine groups, six non-protonated carbons [$\ddot{a}_C$ 40.2 (C-19), 134.4 (C-15), 128.4 (C-16), 127.3 (C-11), 104.1 (C-3), 139.3 (C-2)], and one carbonyl group [$\ddot{a}_C$ 172.1 (C-24)]. The ^1H-^1H COSY correlation systems of compound **3** were similar to those of compound **2** including four systems: H-12/H-13/H-14, H-22/H-23, H-13/H-8 and H-4/H-5/H-10/H-9/H-8/H-7. Detailed analysis of the HMBC correlations with special correlations between H-4 and C-2/C-16/C-3/C-5, between H-4, H-22, H-20, H-21, and C-2 and comparison of its NMR data and optical rotation value $[\alpha]_D^{25}$ with those reported in the literature determined the structure of **3** as fumigaclavine C [11],[12],[13].

Additionally, the remaining compounds were identified as cyclo-(Pro-Phe) (**4**) [14], cyclo-(Pro-Tyr) (**5**) [15],[16], cyclo-(Pro-Ala) (**6**) [17],[18], cyclo-(Pro-Val) (**7**) [16],[19], norharman (**8**) [20], phloretic acid (**9**) [21], and 3-phenylpropanoic acid **(10)** [22] by analysis of NMR data and comparison of their spectroscopic data with those reported in the literature.

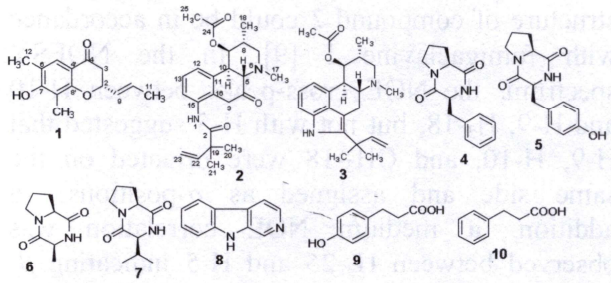

Fig 4. Structure of the isolated compounds **1-10**

All the isolated compounds were evaluated for their antibacterial activity against a range of microorganisms, including *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella enterica* (ATCC13076), *Enterococcus faecalis* (ATCC299212), *Staphylococcus aureus* (ATCC25923) and *Bacillus cereus* (ATCC14579), as well as antiyeast properties against *Candida albicans* (ATCC10231). Except for compounds **4, 5, 7,** and **10**, the other compounds exhibited antimicrobial activity (Table 1). Compounds **2, 3,** and **6** selectively inhibited *E. faecalis* with a MIC value of 64, 128, and 256 µg/mL, respectively which was comparable with the reference compound, *streptomycin* (MIC: 256 µg/mL). Compound **1** exhibited antimicrobial activity against 5 tested strains with MIC values ranging from 16 to 256 µg/mL (Table 1). Compounds **1** and **6** selectively inhibited *C. albicans* with a MIC value of 16 and 2 µg/mL, respectively. Compound **8** selectively inhibited *E. coli* with a MIC value of 128 µg/mL. Finally, compound **9** inhibited *E. faecalis, S. aureus, B. cereus,* and *C. albicans* with MIC values of 256, 256, 256, and 128 µg/mL, respectively.

Table 1. Antimicrobial activity of compounds **1-10**

Compounds	Gram (+)			Gram (-)			Yeast
	E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
	MIC(µg/mL)						
1	128	64	128	-	-	256	16
2	64	-	-	-	-	-	-
3	128	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
6	256	-	-	-	-	-	2
7	-	-	-	-	-	-	-
8	-	-	-	128	-	-	-
9	256	256	256	-	-	-	128
10	-	-	-	-	-	-	-
S	**256**	**256**	**128**	**32**	**256**	**128**	-
C	-	-	-	-	-	-	**32**

Positive controls: S: Streptomycin, C: Cycloheximide; -: inactive (MIC > 256 µg/ml)

4. Conclusion

An investigation into an antimicrobial extract derived from the culture broth of the marine-derived actinomycete *Aspergillus versicolor* M852 led to the isolation of ten compounds (**1-10**). Their structures were identified as (2*R*)-2,3-dihydro-7-hydroxy-6,8-dimethyl-2-[(*E*)-prop-1-enyl]-chroman-4-one (**1**), fumigaclavine I (**2**), fumigaclavine C (**3**), cyclo-(Pro-Phe) (**4**), cyclo-(Pro-Tyr) (**5**), cyclo-(Pro-Ala) (**6**), cyclo-(Pro-Val) (**7**), norharman (**8**), phloretic acid (**9**) and 3-phenylpropanoic acid (**10**). Except for compounds **4**, **5**, **7**, **8,** and **10**, the remaining compounds showed inhibitory activity against one to three Gram-positive strains with MIC values ranging from 64 to 256 µg/mL. Among them, compound **1** was the most potent, inhibiting five out of seven tested microbial strains with MIC values in the range of 16 to 256 µg/mL.

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CHEMICAL COMPOSITION, ANTI-INFLAMMATORY AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OILS FROM *ZANTHOXYLUM MYRIACANTHUM* WALL. EX HOOK. F. FRUITS, LEAVES AND STEMS COLLECTED IN LAM DONG PROVINCE

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Summary

Chemical Composition, Anti-Inflammatory and Cytotoxic Activities of Essential Oils from *Zanthoxylum myriacanthum* Wall. ex Hook. f. Fruits, Leaves and Stems Collected in Lam Dong Province

Essential oils extracted by hydrodistillation from fruits, leaves, and stems of *Z. myriacanthum* collected in Bidoup-Nui Ba National Park, Lam Dong province, Vietnam were investigated for their chemical composition and bioactivity. 51 compounds were identified in the fruit oil (ZMFR), of which *p*-cymen-8-ol (24.9%) and *β*-pinene (24.3%) were the most abundant. The leaf oil (ZML) contained 32 identified constituents, mainly *ρ*-methyl-acetophenone (29.9%), *α*-terpineol (17.0%), *ar*-curcumene (9.5%), (*E*)-carpacin (7.7%), 2-undecanone (6.6%) and 2-tridecanone (5.9%). The stem oil (ZMS) consisted of 23 compounds, the major ones being *ar*-curcumene (44.6%), terpinen-4-ol (13.2%), (*1R,2R,4R*)-4-(2-hydroxypropan-2-yl)-1-methylcyclohexane-1,2-diol (8.8%) and *α*-terpineol (8.4%). ZMFR exhibited moderate NO production inhibition in macrophage RAW 264.7 cells with an IC_{50} value of 42.35 ± 2.63 μg/mL. ZMFR and ZML showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cancer cell lines with the IC_{50} values ranging from 50.97 ± 4.07 to 93.78 ± 3.67μg/mL. ZMS was cytotoxic against HepG2 (IC_{50} = 69.84 ± 4.71 μg/mL) and MCF-7 (IC_{50} = 89.28 ± 5.50 μg/mL), and showed minimal effect on SK-LU-1. The anti-inflammatory and cytotoxic activities of *Z. myriacanthum* essential oils were evaluated and reported for the first time.

Keywords: *Zanthoxylum myriacanthum, Essential oil, GC-MS, Anti-microbial activity, NO inhibition, Cytotoxicity.*