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Chemical profile of the secondary metabolites produced by a deep-sea sediment-derived fungus *Penicillium commune* SD-118*

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Abstract Bioassay-guided fractionation of the crude extract from *Penicillium commune* SD-118, a fungus obtained from a deep-sea sediment sample, resulted in the isolation of a known antibacterial compound, xanthocillin X (**1**), and 14 other known compounds comprising three steroids (**2–4**), two ceramides (**5** and **6**), six aromatic compounds (**7–12**), and three alkaloids (**13–15**). Xanthocillin X (**1**) was isolated for the first time from a marine fungus. In the bioassay, xanthocillin X (**1**) displayed remarkable antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and significant cytotoxicity against MCF-7, HepG2, H460, Hela, Du145, and MDA-MB-231 cell lines. Meleagrin (**15**) exhibited cytotoxicity against HepG2, Hela, Du145, and MDA-MB-231 cell lines. This is the first report of the cytotoxicity of xanthocillin X (**1**).

Keyword: bioguided isolation; deep-sea sediment-derived fungus; *Penicillium commune*; xanthocillin X; antibacterial activity; cytotoxicity

1 INTRODUCTION

Marine-derived fungi have been proven recently to be a potential and prolific source of secondary metabolites with interesting bioactivities that may be useful to identify lead compounds for drug research (Haefner, 2003). In a search for antibacterial and antifungal metabolites from marine-derived fungi, we have isolated more than 400 fungal strains from a variety of marine substrates. Thirty strains derived from deep-sea sediments, algae, and mangrove ecosystems were selected on the basis of their morphological characteristics, and crude extracts from these selected strains were tested for antimicrobial activity. One of the extracts, which exhibited strong inhibition activity against both Gram-positive and Gram-negative bacteria, was obtained from the deep-sea sediment-derived fungus *Penicillium commune* SD-118, which was collected in the South China Sea. Previous studies reported that this species of terrestrial origin primarily synthesized cyclopiazonic acid, rugulovasin, viridicatin, penitrem A, roquefortine, and other mytotoxins, most of which showed strong cytotoxic activity (Larsen et al., 2000; Wagener et al., 1980). Recently, we reported that *P. commune* of marine origin produced comazaphilones, some of which showed potent inhibitory activity against methicillin-resistant *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, and cytotoxic activity against the SW1990 cell line (Gao et al., 2011). Bioactive metabolites of *P. commune* from an

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extremophilic deep-sea environment have not been investigated.

In order to isolate the bioactive compounds, the fungal strain *P. commune* SD-118 was investigated in this study and bioguided fractionation was employed to monitor every isolation step. Fifteen compounds were purified and characterized, which comprised the antimicrobial active compound xanthocillin X (**1**), three steroids (**2–4**), two ceramides (**5** and **6**), six aromatic compounds (**7–12**), and three alkaloids (**13–15**). This paper describes the isolation, structural determination, and bioactivity of these compounds. To our knowledge, this is the first report of xanthocillin X (**1**) obtained from a marine-derived fungus and of its cytotoxic activity.

2 EXPERIMENTAL

2.1 General experimental procedures

The UV spectrum was recorded on a Lengguang Gold 54 spectrometer. NMR spectra were acquired on a Bruker Avance 500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The chemical shift δ is expressed as ppm relative to TMS as the internal standard and coupling constants are expressed in Hz. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory), reversed-phase silica gel C18 (40–63 μm, Merck), and Sephadex LH-20 gel (Pharmacia) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates were used for TLC (Qingdao Haiyang Chemical Factory). Analytical and semi-preparative HPLC were performed using an Dionex Acclaim ODS column (4.6 mm × 250 mm, 5 μm) and SinoChrom ODS-BP column (10 mm × 300 mm, 10 μm), respectively.

2.2 Fungal material

The fungus was isolated by a serial dilution method on potato dextrose agar (PDA) plates from a deep-sea sediment sample collected from the South China Sea (at a depth of 1 188 m), in September 2008. Fungal identification was carried out by amplification and sequence analysis of 5.8S rDNA and the internal transcribed spacer (ITS) region as reported previously (Wang et al., 2006). The sequence data derived from the fungal strain was deposited in GenBank (accession No. HQ652873). A nucleotide BLAST search revealed that the sequence was 100% identical to a sequence for *Penicillium commune* (accession No. FJ499451). The fungal strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences.

2.3 Fermentation and preparation of extract

For metabolite investigation, the strain *P. commune* SD-118 was recovered in Petri dishes with PDA medium in seawater at 28°C for 3 d. Then agar plugs were inoculated into 500 mL Erlenmeyer flasks that each contained 150 mL Wickerham medium (0.45 g malt extract, 0.75 g peptone, 1.50 g glucose, 150 mL filtered seawater) whose pH was adjusted to 7.0 before sterilization. The cultures were incubated on a rotary shaker at 28°C for 3 d, with a rotational speed of 180 r/min, to yield seed liquid. After inoculation with 10 mL of the seed liquid, 100 Erlenmeyer flasks that each contained liquid fermentation medium composed of 6.0 g mannitol, 6.0 g maltose, 3.0 g monosodium glutamate, 0.9 g yeast extract, 3.0 g glucose, 0.3 g corn steep liquor, 0.09 g $MgSO_4 \cdot 7H_2O$, 0.15 g KH_2PO_4, 0.15 g L-tryptophan, and 300 mL filtered seawater were statically cultured for 33 d at room temperature. After incubation, EtOAc was added to sterilize the fermented broth, and then the mycelia were separated from the culture broth by filtration. The broth was extracted repeatedly with EtOAc (200 mL per flask) to yield an extract, and the mycelium was air-dried and soaked three times with acetone/water (4:1, *v/v*) for 3 d. The acetone/water extract was evaporated to remove acetone under vacuum and then extracted with EtOAc to afford another extract. The combined extraction (27 g) was fractionated by silica gel vacuum liquid chromatography (VLC) using petroleum/EtOAc (from 1:0 to 1:1) and $CHCl_3$/MeOH (from 20:1 to 0:1) gradient elution to afford 12 fractions (Fr.1–Fr.12).

2.4 Bioguided isolation

Antibacterial activity of each fraction (Fr.1–Fr.12) against *Staphylococcus aureus* and *Escherichia coli* was screened by a filter-paper disk method at 200 mg/mL compared with that of the positive control chloramphenicol at 4 mg/mL. The fraction (Fr.8), which showed strong antibacterial activity, was further subjected to reversed-phase C18 column chromatography elution with a MeOH/H_2O gradient solvent system (from 20% to 100% MeOH in water) to yield five subfractions (Fr.8-1 to Fr.8-5). Each subfraction was analyzed subsequently by HPLC (gradient elution: 0–5 min, 20% MeOH; 5–40 min, 20% to 80% MeOH; 40–50 min, 100% MeOH) and tested for antibacterial activity using the same

method as described above. The bioactive fractions Fr.8-4 and Fr.8-5 were combined and purified by semi-preparative HPLC (70% MeOH, 3.0 mL/min) to obtain compound **1** (56.5 mg). Fr.8-2 was further purified by semi-preparative HPLC (100% MeOH, 3.0 mL/min) to afford compound **6** (16.1 mg). Fr.8-3 was subjected to semi-preparative HPLC (30% CH_3CN, 3.0 mL/min) to yield compounds **11** (8.5 mg) and **14** (3.0 mg). Compound **2** (7.9 mg) was obtained from Fr.3 by recrystallization from chloroform. Fr.5 and Fr.6 were combined and separated by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to yield two subfractions (Fr.5/6-1 and Fr.5/6-2). Fr.5/6-1 was purified further by reversed-phase C18 CC (90% MeOH) to yield compound **4** (16.6 mg). Fr.5/6-2 was subjected to semi-preparative HPLC (20% CH_3CN, 3.0 mL/min) to obtain compounds **8** (9.6 mg) and **10** (10.4 mg). Fr.7 was purified by silica gel CC, eluted with a $CHCl_3$/MeOH gradient (from 100:1 to 1:1) and Sephadex LH-20 (100% MeOH), to obtain compounds **3** (7.5 mg), **5** (18.7 mg), **7** (14.7 mg), **9** (7.2 mg), **13** (3.7 mg), and **15** (27.9 mg). Fr.9 was fractionated into three parts (Fr.9-1 to Fr.9-3) by reversed-phase C18 CC eluted with a 15% to 50% aqueous MeOH gradient. Fr.9-2 was purified by semi-preparative HPLC (20% MeOH, 3.0 mL/min) to yield compound **12** (5.6 mg).

2.5 Antimicrobial assay

Antibacterial activity against two bacteria (*Staphylococcus aureus* and *Escherichia coli*), and antifungal activity against five plant-pathogenic fungi (*Alternaria brassicae*, *Fusarium oxysporium* f. sp. *vasinfectum*, *Coniella diplodiella*, *Physalospora piricola*, and *Aspergillus niger*), were evaluated *in vitro* by a standard disk diffusion method (Al-Burtamani et al., 2005) and broth microdilution method (Shen et al., 2002). Chloramphenicol and amphotericin B were used as antibacterial and antifungal positive controls, respectively.

2.6 Cytotoxicity assay

Cytotoxic activity against MCF-7 (human breast adenocarcinoma), SW1990 (human pancreatic cancer), HepG2 (human hepatocellular liver carcinoma), NCI-H460 (human non-small cell lung cancer), A549 (human lung adenocarcinoma), HeLa (human epithelial carcinoma), DU145 (human prostate carcinoma), and MDA-MB-231 (human breast adenocarcinoma) cell lines were determined following the method of Bergeron et al. (1984). Fluorouracil was used as a positive control.

3 SPECTRAL DATA

Xanthocillin X (1)

Yellow amorphous powder, $C_{18}H_{12}N_2O_2$; UV (MeOH) λ_{max} (log ε) 234 (4.20), 363 (4.72); Negative ESIMS *m/z* 287 $[M-H]^-$, 574 $[2M-2H]^-$; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 7.05 (2H, s, H-2, H-2′), 7.74 (4H, d, J=8.8 Hz, H-4, H-4′, H-8, H-8′), 6.90 (4H, d, J=8.7 Hz, H-5, H-5′, H-7, H-7′), 10.22 (2H, br s, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 114.7 (C-1, C-1′, C), 127.5 (C-2, C-2′, CH), 122.9 (C-3, C-3′, C), 131.7 (C-4, C-4′, C-8, C-8′, CH), 115.9 (C-5, C-5′, C-7, C-7′, CH), 159.5 (C-6, C-6′, C), 172.7 (C-9, C-9′, C).

(22*E*,24*R*)-ergosta-5,7,22-trien-3*β*-ol (2)

Colorless needle (chloroform), $C_{28}H_{44}O$; EIMS (70 eV) *m/z* (rel. int.) 396 $[M]^+$ (69), 378 $[M-H_2O]^+$ (10), 363 $[M-H_2O-CH_3]^+$ (81), 337 $[M-H_2O-C_3H_5]$ (35); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 3.64 (1H, m, H-3), 2.47 (1H, m, H-4a), 2.28 (1H, m, H-4b), 5.57 (1H, dd, J=5.6, 2.4 Hz, H-6), 5.38 (1H, dt, J=5.3, 2.6 Hz, H-7), 0.95 (3H, s, H-18), 0.63 (3H, s, H-19), 1.03 (3H, d, J=6.7 Hz, H-21), 5.23 (1H, dd, J=15.2, 7.1 Hz, H-22), 5.17 (1H, dd, J=15.3, 7.7 Hz, H-23), 0.82 (3H, d, J=7.2 Hz, H-26), 0.84 (3H, d, J=7.2 Hz, H-27), 0.92 (3H, d, J=6.9 Hz, H-28). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 38.4 (C-1, CH_2), 32.0 (C-2, CH_2), 70.5 (C-3, CH), 40.8 (C-4, CH_2), 139.8 (C-5, C), 119.6 (C-6, CH), 116.3 (C-7, CH), 141.3 (C-8, C), 46.3 (C-9, CH), 37.1 (C-10, C), 21.1 (C-11, CH_2), 39.1 (C-12, CH_2), 42.8 (C-13, C), 54.6 (C-14, CH), 23.0 (C-15, CH_2), 28.3 (C-16, CH_2), 55.8 (C-17, CH), 12.1 (C-18, CH_3), 16.3 (C-19, CH_3), 40.4 (C-20, CH), 21.1 (C-21, CH_3), 135.6 (C-22, CH), 132.0 (C-23, CH), 42.8 (C-24, CH), 33.1 (C-25, CH), 19.9 (C-26, CH_3), 19.6 (C-27, CH_3), 17.6 (C-28, CH_3).

(22*E*,24*R*)-ergosta-7,22-dien-3*β*,5*α*,6*β*-triol (3)

Colorless needle (chloroform), $C_{28}H_{46}O_3$; EIMS (70 eV) *m/z* (rel. int.) 430 $[M]^+$ (2.5), 412 $[M-H_2O]^+$ (50), 394 $[M-2H_2O]^+$ (35), 383 $[M-H_2O-C_2H_5]^+$ (38), 379 $[M-2H_2O-CH_3]^+$ (60); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 4.08 (1H, m, H-3), 3.62 (1H, d, J=5.2 Hz, H-6), 5.35 (1H, m, H-7), 0.60 (3H, s, H-18), 1.08 (3H, s, H-19), 1.02 (3H, d, J=6.6 Hz, H-21), 5.23 (1H, dd, J=15.3, 7.4 Hz, H-22), 5.16 (1H, dd, J=15.3, 8.2 Hz, H-23), 0.82 (3H, d, J=7.0 Hz, H-26), 0.84 (3H, d, J=7.6 Hz, H-27), 0.91 (3H, d, J=6.9 Hz, H-28). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 30.9 (C-1, CH_2), 29.7 (C-2, CH_2), 67.7

(C-3, CH), 39.5 (C-4, CH_2), 76.0 (C-5, C), 73.7 (C-6, CH), 117.6 (C-7, CH), 144.0 (C-8, C), 43.5 (C-9, CH), 37.2 (C-10, C), 22.1 (C-11, CH_2), 39.2 (C-12, CH_2), 43.8 (C-13, C), 54.8 (C-14, CH), 22.9 (C-15, CH_2), 27.9 (C-16, CH_2), 56.0 (C-17, CH), 12.3 (C-18, CH_3), 18.8 (C-19, CH_3), 40.4 (C-20, CH), 21.1 (C-21, CH_3), 135.4 (C-22, CH), 132.2 (C-23, CH), 42.8 (C-24, CH), 33.1 (C-25, CH), 19.6 (C-26, CH_3), 19.9 (C-27, CH_3), 17.6 (C-28, CH_3).

(22*E*,24*R*)-5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-ol (4)

White amorphous powder, $C_{28}H_{44}O_3$; EIMS (70 eV) *m/z* (rel. int.) 428 $[M]^+$ (1.7), 396 $[M - O_2]^+$ (100), 363 $[M - O_2 - H_2O - CH_3]^+$ (49), 337 $[M - O_2 - H_2O - C_3H_5]^+$ (26); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 3.96 (1H, m, H-3), 6.50 (1H, d, *J*=8.5 Hz, H-6), 6.24 (1H, d, *J*=8.5 Hz, H-7), 0.81 (3H, s, H-18), 0.88 (3H, s, H-19), 0.99 (3H, d, *J*=6.6 Hz, H-21), 5.14 (1H, dd, *J*=15.3, 8.3 Hz, H-22), 5.22 (1H, dd, *J*=15.3, 7.6 Hz, H-23), 0.81 (3H, d, *J*=6.9 Hz, H-26), 0.83 (3H, d, *J*=7.1 Hz, H-27), 0.90 (3H, d, *J*=6.9 Hz, H-28). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 34.7 (C-1, CH_2), 30.1 (C-2, CH_2), 66.4 (C-3, CH), 39.4 (C-4, CH_2), 82.1 (C-5, C), 135.2 (C-6, CH), 130.7 (C-7, CH), 79.4 (C-8, C), 51.1 (C-9, CH), 37.0 (C-10, C), 20.6 (C-11, CH_2), 36.9 (C-12, CH_2), 44.6 (C-13, C), 51.7 (C-14, CH), 23.4 (C-15, CH_2), 28.6 (C-16, CH_2), 56.2 (C-17, CH), 12.9 (C-18, CH_3), 18.1 (C-19, CH_3), 39.7 (C-20, CH), 20.9 (C-21, CH_3), 135.4 (C-22, CH), 132.3 (C-23, CH), 42.8 (C-24, CH), 33.1 (C-25, CH), 19.6 (C-26, CH_3), 19.9 (C-27, CH_3), 17.5 (C-28, CH_3).

Asperamide A (5)

White amorphous powder, $C_{37}H_{69}NO_4$; Positive FABMS *m/z* 592 $[M + H]^+$, 574 $[M + H - H_2O]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 3.73 (1H, m, H-1a), 3.88 (1H, m, H-1b), 3.87 (1H, m, H-2), 4.26 (1H, br s, H-3), 5.50 (1H, dd, *J*=15.7, 6.3 Hz, H-4), 5.78 (1H, m, H-5), 2.07 (2H, m, H-6), 2.06 (2H, m, H-7), 5.09 (1H, t, *J*=6.1 Hz, H-8), 1.94 (2H, t, *J*=7.6 Hz, H-10), 1.36 (2H, m, H-11), 1.31–1.25 (16H, br s, H-12–H-19), 0.87 (3H, t, *J*=6.9 Hz, H-20), 1.58 (3H, s, H-21), 4.54 (1H, d, *J*=6.5 Hz, H-2′), 5.53 (1H, dd, *J*=17.4, 6.6 Hz, H-3′), 5.88 (1H, dt, *J*=15.3, 7.2 Hz, H-4′), 2.04 (2H, m, H-5′), 1.36 (2H, m, H-6′), 1.31–1.25 (18H, br s, H-7′–H-15′), 0.87 (3H, t, *J*=6.9 Hz, H-16′), 7.17 (1H, d, *J*=7.6 Hz, NH), 3.32 (3H, br s, OH-1, OH-3, OH-2′). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 61.9 (C-1, CH_2), 54.7 (C-2, CH), 74.0 (C-3, CH), 128.7 (C-4, CH), 134.0 (C-5, CH), 32.5 (C-6, CH_2), 27.6 (C-7, CH_2), 123.1 (C-8, CH), 136.2 (C-9, C), 39.7 (C-10, CH_2), 28.0 (C-11, CH_2), 29.7–29.2 (C-12–C-17, CH_2), 31.9 (C-18, CH_2), 22.7 (C-19, CH_2), 14.1 (C-20, CH_3), 16.0 (C-21, CH_3), 173.6 (C-1′, C), 73.2 (C-2′, CH), 127.0 (C-3′, CH), 135.9 (C-4′, CH), 32.3 (C-5′, CH_2), 29.0 (C-6′, CH_2), 29.7–29.2 (C-7′–C-13′, CH_2), 31.9 (C-14′, CH_2), 22.7 (C-15′, CH_2), 14.1 (C-16′, CH_3).

Asperamide B (6)

White amorphous powder, $C_{43}H_{79}NO_9$; ESIMS *m/z* 754 $[M + H]^+$, 736 $[M + H - H_2O]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ_H: 4.14 (1H, dd, *J*=9.3, 5.4 Hz, H-1a), 3.72 (1H, dd, *J*=10.2, 3.2 Hz, H-1b), 3.89 (1H, m, H-2), 4.13 (1H, dd, *J*=7.1, 5.5 Hz, H-3), 5.48 (1H, dd, *J*=16.0, 7.4 Hz, H-4), 5.73 (1H, dt, *J*=15.3, 6.6, H-5), 2.08 (2H, m, H-6), 2.05 (2H, m, H-7), 5.16 (1H, t, *J*=5.7 Hz, H-8), 1.98 (2H, t, *J*=7.3 Hz, H-10), 1.41 (2H, m, H-11), 1.36–1.31 (16H, br s, H-12–H-19), 0.91 (3H, t, *J*=7.0 Hz, H-20), 1.60 (3H, s, H-21), 4.43 (1H, d, *J*=5.8 Hz, H-2′), 5.48 (1H, dd, *J*=16.3, 6.0, H-3′), 5.85 (1H, dd, *J*=15.3, 7.7 Hz, H-4′), 2.03 (2H, m, H-5′), 1.42 (2H, m, H-6′), 1.36–1.31 (18H, br s, H-7′–H-15′), 0.93 (3H, t, *J*=7.0 Hz, H-16′), 4.28 (1H, d, *J*=7.7 Hz, H-1″), 3.20 (1H, t, *J*=8.1 Hz, H-2″), 3.36 (1H, t, *J*=8.8 Hz, H-3″), 3.31 (1H, m, H-4″), 3.30 (1H, m, H-5″), 3.89 (1H, d, *J*=11.7 Hz, H-6″a), 3.71 (1H, dd, *J*=11.7, 4.3 Hz, H-6″b); ^{13}C NMR (125 MHz, CD_3OD) δ_C: 69.7 (C-1, CH_2), 54.7 (C-2, CH), 72.9 (C-3, CH), 131.0 (C-4, CH), 134.5 (C-5, CH), 33.0 (C-6, CH_2), 28.8 (C-7, CH_2), 124.9 (C-8, CH), 136.8 (C-9, C), 40.8 (C-10, CH_2), 29.1 (C-11, CH_2), 30.2–30.8 (C-12–C-17, CH_2), 33.1 (C-18, CH_2), 23.7 (C-19, CH_2), 14.4 (C-20, CH_3), 16.2 (C-21, CH_3), 175.5 (C-1′, C), 74.1 (C-2′, CH), 129.1 (C-3′, CH), 134.8 (C-4′, CH), 33.4 (C-5′, CH_2), 30.2–30.8 (C-6′–C-13′, CH_2), 33.1 (C-14′, CH_2), 23.7 (C-15′, CH_2), 14.4 (C-16′, CH_3), 104.7 (C-1″, CH), 75.0 (C-2″, CH), 78.0 (C-3″, CH), 71.6 (C-4″, CH), 78.0 (C-5″, CH), 62.7 (C-6″, CH_2).

Chrysogine (7)

White powder, $C_{10}H_{10}N_2O_2$; Positive ESI-TOF-MS *m/z* 191 $[M + H]^+$, 213 $[M + Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 8.10 (1H, d, *J*=7.6 Hz, H-3), 7.48 (1H, t, *J*=7.5 Hz, H-4), 7.79 (1H, t, *J*=7.6 Hz, H-5), 7.63 (1H, d, *J*=8.1 Hz, H-6), 4.59 (1H, m, H-9), 1.43 (3H, d, *J*=6.6 Hz, H-10), 5.65 (1H, d, *J*=7.6 Hz, OH-9), 11.79 (1H, br s, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 161.4 (C-1, C), 121.2 (C-2, C), 125.7 (C-3, CH), 126.2 (C-4, CH), 134.3 (C-5, CH), 126.9 (C-6, CH), 148.4 (C-7, C), 159.6 (C-8, C), 67.1 (C-9, CH), 21.5 (C-10, CH_3).

Methyl 2-(*N*-(2-hydroxyphenyl)carbamoy)acetate (8)

White powder, $C_{10}H_{11}NO_4$; EIMS (70 eV) *m/z* (rel. int.) 209 $[M]^+$ (100), 191 $[M - H_2O]^+$ (13), 177 $[M - CH_3OH]^+$ (33), 135 $[M - CH_3OH - CH_2CO]^+$ (35); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 6.87 (1H, d, J=7.9 Hz, H-3), 6.92 (1H, t, J=7.6 Hz, H-4), 6.75 (1H, t, J=7.4 Hz, H-5), 7.87 (1H, d, J=8.0 Hz, H-6), 3.60 (2H, s, H-9), 3.65 (3H, s, 10-OCH_3), 9.93 (1H, br s, OH), 9.50 (1H, br s, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 126.0 (C-1, C), 148.9 (C-2, C), 115.3 (C-3, CH), 124.4 (C-4, CH), 118.8 (C-5, CH), 121.5 (C-6, CH), 164.0 (C-8, C), 42.8 (C-9, CH_2), 168.5 (C-10, C), 51.8 (10-OCH_3).

***N*-(2-Hydroxypropanoyl)-2-aminobenzoic acid amide (9)**

White powder, $C_{10}H_{12}N_2O_3$; EIMS (70 eV) *m/z* (rel. int.) 208 $[M]^+$ (23), 163 $[M - C_2H_5O]^+$ (98), 146 $[M - C_2H_5O - NH_3]^+$ (100), 119 $[M - C_2H_5O - CONH_2]^+$ (67); ^{1}H NMR (500 MHz, CD_3OD) δ_H: 8.53 (1H, dd, J=8.4, 1.0 Hz, H-3), 7.49 (1H, m, H-4), 7.16 (1H, td, J=7.6, 1.1 Hz, H-5), 7.73 (1H, dd, J=7.9, 1.4 Hz, H-6), 4.23 (1H, q, J=6.9 Hz, H-9), 1.43 (3H, d, J=6.9 Hz, H-10). ^{13}C NMR (125 MHz, CD_3OD) δ_C: 122.9 (C-1, C), 139.6 (C-2, C), 122.2 (C-3, CH), 133.2 (C-4, CH), 124.5 (C-5, CH), 129.4 (C-6, CH), 173.3 (C-7, C), 176.6 (C-8, C), 69.8 (C-9, CH), 21.1 (C-10, CH_3).

***N*-(2-Hydroxyphenyl)acetamide (10)**

White powder, $C_8H_9NO_2$; EIMS (70 eV) *m/z* (rel. int.) 151 $[M]^+$ (88), 109 $[M - CH_2CO]^+$ (100); ^{1}H NMR (500 MHz, acetone-d_6) δ_H: 6.89 (1H, dd, J=8.0, 1.2 Hz, H-3), 7.02 (1H, td, J=7.2, 1.5 Hz, H-4), 6.80 (1H, td, J=7.1, 1.3 Hz, H-5), 7.38 (1H, d, J=7.9 Hz, H-6), 2.20 (3H, s, NHAc), 9.26 (1H, br s, OH), 9.38 (1H, br s, NH). ^{13}C NMR (125 MHz, acetone-d_6) δ_C: 127.8 (C-1, C), 149.5 (C-2, C), 119.0 (C-3, CH), 126.6 (C-4, CH), 120.5 (C-5, CH), 122.8 (C-6, CH), 171.2 (NHAc, C), 23.5 (NHAc, CH_3).

4-Hydroxybenzaldehyde (11)

White powder, $C_7H_6O_2$; EIMS (70 eV) *m/z* (rel. int.) 122 $[M]^+$ (87), 121 $[M - H]^+$ (100), 93 $[M - CHO]^+$ (36); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 7.74 (2H, d, J=8.5 Hz, H-2, H-6), 6.89 (2H, d, J=8.5 Hz, H-3, H-5), 9.76 (1H, s, H-7). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 127.9 (C-1, C), 132.0 (C-2, C-6, CH), 116.0 (C-3, C-5, CH), 164.1 (C-4, C), 190.6 (C-7, CH).

***N*-Acetyldopamine (12)**

Brown oil, $C_{10}H_{13}NO_3$; EIMS (70 eV) *m/z* (rel. int.) 195 $[M]^+$ (25), 136 $[M - AcNH_2]^+$ (100), 123 $[M - AcNH{=}CH_2]^+$ (93); ^{1}H NMR (500 MHz, CD_3OD) δ_H: 6.64 (1H, d, J=1.4 Hz, H-2), 6.67 (1H, d, J=8.0 Hz, H-5), 6.52 (1H, dd, J=7.9, 1.5 Hz, H-6), 3.31 (2H, overlap, H-1′), 2.62 (2H, t, J=7.4 Hz, H-2′), 1.90 (3H, s, NHAc). ^{13}C NMR (125 MHz, CD_3OD) δ_C: 132.1 (C-1, C), 116.4 (C-2, CH), 144.8 (C-3, C), 146.2 (C-4, C), 116.9 (C-5, CH), 121.0 (C-6, CH), 42.3 (C-1′, CH_2), 35.9 (C-2′, CH_2), 173.2 (NHAc, C), 22.5 (NHAc, CH_3).

Methyl 2-([2-(1H-indol-3-yl)ethyl]carbamoyl)acetate (13)

Yellow oil, $C_{14}H_{16}N_2O_3$; EIMS (70 eV) *m/z* (rel. int.) 260 $[M]^+$ (47), 229 $[M - OCH_3]^+$ (6), 187 $[M - OCH_3 - CH_2CO]^+$ (10), 143 $[M - OCH_3 - CH_2CO - CONH_2]^+$ (100), 130 $[M - OCH_3 - CH_2CO - CONH{=}CH_2]^+$ (97); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 10.84 (1H, br s, NH-1), 7.15 (1H, d, J=1.9 Hz, H-2), 7.52 (1H, d, J=7.8 Hz, H-4), 6.98 (1H, td, J=7.4 Hz, 1.0 Hz, H-5), 7.06 (1H, td, J=7.5 Hz, 1.1 Hz, H-6), 7.33 (1H, d, J=8.1 Hz, H-7), 2.82 (2H, t, J=7.4 Hz, H-8), 3.35 (2H, m, H-9), 8.21 (1H, t, J=5.3 Hz, NH-10), 3.23 (2H, s, H-12), 3.61 (3H, s, 13-OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 123.1 (C-2, CH), 112.1 (C-3, C), 127.7 (C-3a, C), 118.6 (C-4, CH), 118.7 (C-5, CH), 121.3 (C-6, CH), 111.8 (C-7, CH), 136.7 (C-7a, C), 25.4 (C-8, CH_2), 40.1 (C-9, CH_2), 165.4 (C-11, C), 42.8 (C-12, CH_2), 168.9 (C-13, C), 52.2 (13-OCH_3).

N2′-Acetyltryptophan methyl ester (14)

Yellow oil, $C_{14}H_{16}N_2O_3$; EIMS (70 eV) *m/z* (rel. int.) 260 $[M]^+$ (26), 201 $[M - COOCH_3]^+$ (82); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 10.87 (1H, br s, NH-1), 7.14 (1H, d, J=2.2 Hz, H-2), 7.48 (1H, d, J=7.8 Hz, H-4), 6.98 (1H, t, J=7.4 Hz, H-5), 7.06 (1H, t, J=7.6 Hz, H-6), 7.33 (1H, d, J=8.4 Hz, H-7), 3.01 (1H, dd, J=14.5, 8.5 Hz, H-8a), 3.14 (1H, dd, J=14.7, 8.0 Hz, H-8b), 4.47 (1H, dd, J=13.7, 7.8 Hz, H-9), 1.81 (3H, s, NHAc), 3.57 (3H, s, 10-OCH_3), 8.30 (1H, d, J=7.2 Hz, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 123.5 (C-2, CH), 109.4 (C-3, C), 127.0 (C-3a, C), 117.9 (C-4, CH), 118.3 (C-5, CH), 120.8 (C-6, CH), 111.3 (C-7, CH), 136.0 (C-7a, C), 27.0 (C-8, CH_2), 53.0 (C-9, CH), 172.4 (C-10, C), 169.2 (NHAc, C), 22.2 (NHAc, CH_3), 51.6 (10-OCH_3).

Meleagrin (15)

Yellow amorphous powder, $C_{23}H_{23}N_5O_4$; EIMS (70 eV) *m/z* (rel. int.) 433 $[M]^+$ (28), 402 $[M - OMe]^+$ (14); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 7.54 (1H, d, J=7.7 Hz, H-4), 7.04 (1H, t, J=7.6 Hz, H-5), 7.26 (1H, t, J=7.6 Hz, H-6), 6.97 (1H, d, J=7.8 Hz, H-7), 5.25 (1H, s, H-8), 10.06 (1H, s, NH-14), 8.19 (1H, s, H-15), 12.89 (1H, s, NH-17 or NH-19), 7.76 (1H, s, H-18), 7.37 (1H, s, H-20), 6.01 (1H, s, H-22), 4.98 (2H, m, H-23), 1.20 (6H, s, H-24, H-25), 3.66 (3H, s, 1-OCH_3), 9.21 (1H, s, OH-9). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 101.2 (C-2, C), 52.2 (C-3, C), 125.9 (C-3a, C), 124.6 (C-4, CH), 123.0 (C-5, CH), 127.8 (C-6, CH), 111.4 (C-7, CH), 146.2 (C-7a, C), 109.1 (C-8, CH), 142.8 (C-9, C), 158.5 (C-10, C), 123.6 (C-12, C), 164.7 (C-13, C), 106.7 (C-15, CH), 125.5 (C-16, C), 137.5 (C-18, CH), 134.1 (C-20, CH), 41.8 (C-21, C), 143.2 (C-22, CH), 112.8 (C-23, CH_2), 24.0 (C-24, CH_3), 23.2 (C-25, CH_3), 64.7 (1-OCH_3).

4 RESULT AND DISCUSSION

4.1 Bioguided isolation and structural elucidation of xanthocillin X (1)

In order to identify the bioactive compounds, all of the fractions (Fr.1–Fr.12) eluted with a gradient solvent system from VLC were screened for antibacterial activity with a filter-paper disk method. The bioactive components that inhibited *E. coli* growth were mainly attributable to the Fr.6–Fr.11 fractions (eluted with PE/EtOAc=1:1; $CHCl_3$/MeOH=20:1, 10:1, 5:1, 2:1, and 1:1) with inhibition zone diameters of 14, 18, 21, 15, 16, and 10 mm, respectively. These fractions also showed strong inhibitory activity against *S. aureus* with 12, 15, 21, 17, 19, and 11 mm inhibition zones, respectively. The Fr.8 fraction was the most active toward *S. aureus* and *E. coli*, and was chosen for subsequent bioguided isolation. Therefore, Fr.8 was further fractionated by reversed-phase C18 CC eluted with a solvent gradient of 20%, 40%, 60%, 80%, and 100% MeOH in water to yield five subfractions (Fr.8-1 to Fr.8-5); each subfraction was investigated for antibacterial activity and subjected to HPLC analysis. The Fr.8-4 and Fr.8-5 were bioactive subfractions that both induced inhibition zones of 15 mm toward *E. coli* and 14 mm toward *S. aureus*. The two bioactive subfractions had almost identical HPLC profiles, which showed a main peak with retention time at 37.42 min. On the basis of these results, Fr.8-4 and Fr.8-5 were combined and purified by semi-preparative HPLC (70% MeOH, 3.0 mL/min) to yield compound **1**. This compound was also isolated from other bioactive fractions (Fr.6–Fr.7 and Fr.9–Fr.11). The overall yield of this compound produced by the fungus in fermentation liquid was 361.55 mg/L. Antimicrobial assays of all of the purified compounds confirmed that compound **1** was the main bioactive compound responsible for antimicrobial activity in the bioguided isolation. The antibacterial bioguided isolation is illustrated in Fig.1.

Compound **1** was obtained as a yellow amorphous powder. After spraying with anisaldehyde reagent followed by heating, the compound displayed a yellow tailed spot on a TLC system developed with $CHCl_3$/MeOH (20:1, *v/v*, with 0.2% acetic acid). The low-resolution negative ESIMS displayed ion peaks at *m/z* 287 $[M - H]^-$ and 574 $[2M - 2H]^-$. The ^{1}H- and ^{13}C-NMR spectra of **1** showed six proton and nine carbon resonances. Further analysis of ^{1}H-, ^{13}C-, DEPT, and HSQC data indicated the presence of one isocyanide group at δ_C 172.7, three quaternary carbons at δ_C 159.5, 122.9, and 114.7, one olefinic methine at δ_C 127.5 (δ_H 7.05, s, H-2), and two aromatic methines at δ_C 131.7 (δ_H 7.74, d, J=8.8 Hz, H-4, H-8) and 115.9 (δ_H 6.90, d, J=8.7 Hz, H-5, H-7). These resonances indicated the symmetrical nature in the structure of **1**. The ^{1}H-^{1}H COSY correlation from H-4/H-8 to H-5/H-7 and HMBC correlations from H-4/H-8 to C-2 and C-6, and from H-5/H-7 to C-3 and C-6 suggested the presence of a *para*-hydroxyphenyl group and connection between C-2 and C-3. The single olefinic proton H-2 showed HMBC cross-peaks with C-1 and C-4/C-8, which confirmed the connection of the ethylenic unit to aromatic and isocyanide groups, respectively. On the basis of the above evidence and comparison with published data (Vesonder, 1979), compound **1** was identified as xanthocillin X. This is the first report of the isolation of xanthocillin X from a marine-derived fungus.

Xanthocillin X (**1**) is a naturally occurring isocyanide compound first obtained from *P. notatum* (Rothe, 1950), *P. chrysogenum* (de la Campa et al., 2007) and *P. expansum* (Kozlovsky et al., 2004). It is noteworthy that xanthocillin X (**1**) and its analogues (e.g. monomethyl ether derivative, dimethyl ether derivative, and methoxyxanthocillin X dimethyl ether) show not only broad antibacterial spectra but also a wide range of biological activities, such as antiviral action, aromatase inhibitory action, antitumor and anthelmintic activity, prostaglandin

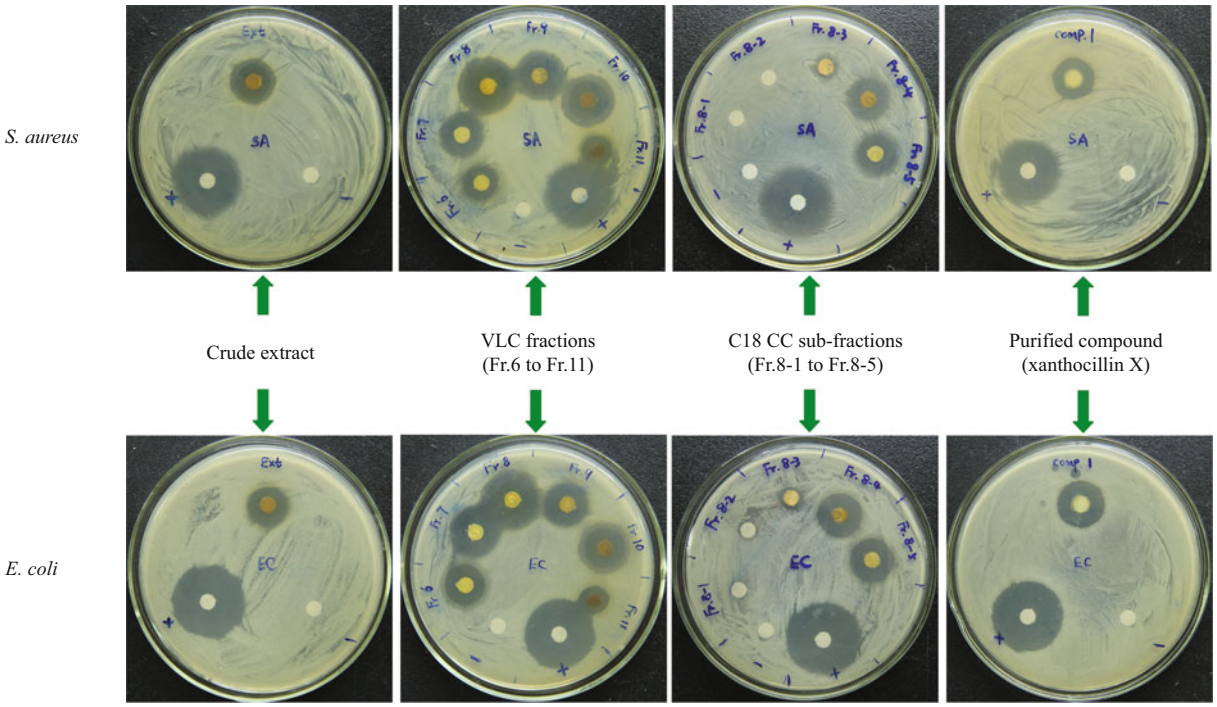

Fig.1 Antibacterial evaluation of crude extract, column fractions, and purified compounds (xanthocillin X) from *Penicillium commune* SD-118

+: positive control, chloramphenicol, 20 μg/disk; crude extract and column fractions were tested at 1.0 mg/disk; purified compounds were tested at 100 μg/disk.

synthesis inhibitory action, platelet aggregation inhibitory action, and thrombopoietin receptor agonistic action (Miyaji et al., 2005). The structure-activity relationship of xanthocillin X (**1**) and its derivatives indicated that the isocyanide group was not needed to sustain biological activity as a thrombopoietin receptor agonist. Removal of one isocyanide group did not destroy activity but reduced efficacy (Yamaguchi et al., 2006). The structure-activity relationship of xanthocillin X (**1**) as an antibacterial agent has not been reported previously and therefore deserves further study.

4.2 Other chemical constituents of *P. commune* SD-118

In addition to compound **1**, the structures of compounds **2–15** were determined by detailed MS and NMR spectral analysis, and by comparison with published data. These compounds were identified as (22*E*,24*R*)-ergosta-5,7,22-trien-3*β*-ol (**2**) (Adler et al., 1977; Smith, 1977), (22*E*,24*R*)-ergosta-7,22-dien-3*β*,5*α*,6*β*-triol (**3**) (Cafieri et al., 1985), (22*E*,24*R*)-5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-ol (**4**) (Yue et al., 2001), asperamide A (**5**) (Zhang et al., 2007), asperamide B (**6**) (Zhang et al., 2007), chrysogine (**7**) (Hikino et al., 1973; Kettering et al., 2004), methyl 2-(*N*-(2-hydroxyphenyl)carbamoy)acetate (**8**) (TimTec Compound Libraries, 2010), *N*-(2-hydroxypropanoyl)-2-aminobenzoic acid amide (**9**) (Dai et al., 1993), *N*-(2-hydroxyphenyl)acetamide (**10**) (Rahaim Jr et al., 2006), 4-hydroxybenzaldehyde (**11**) (Andersen et al., 1974), *N*-acetyldopamine (**12**) (Noda et al., 2000), methyl 2-([2-(1H-indol-3-yl)ethyl]carbamoyl)acetate (**13**) (Franzén et al., 2009), N2′-acetyltryptophan methyl ester (**14**) (Amir-Heidari and Micklefield, 2007), and meleagrin (**15**) (Du et al., 2009). The chemical structures of compounds **1–15** are shown in Fig.2 and the peaks of the major compounds in the crude extract were assigned in the HPLC profile (Fig.3).

4.3 In-vitro antimicrobial activity and cytotoxicity

All isolated compounds were tested for antimicrobial activity toward two bacteria (*S. aureus* and *E. coli*) and five plant-pathogenic fungi (*A. brassicae*, *F. oxysporium*, *C. diplodiella*, *P. piricola*, and *A. niger*) with disk diffusion method. Compound **1** exhibited strong antibacterial activity against both Gram-positive (*S. aureus*) and

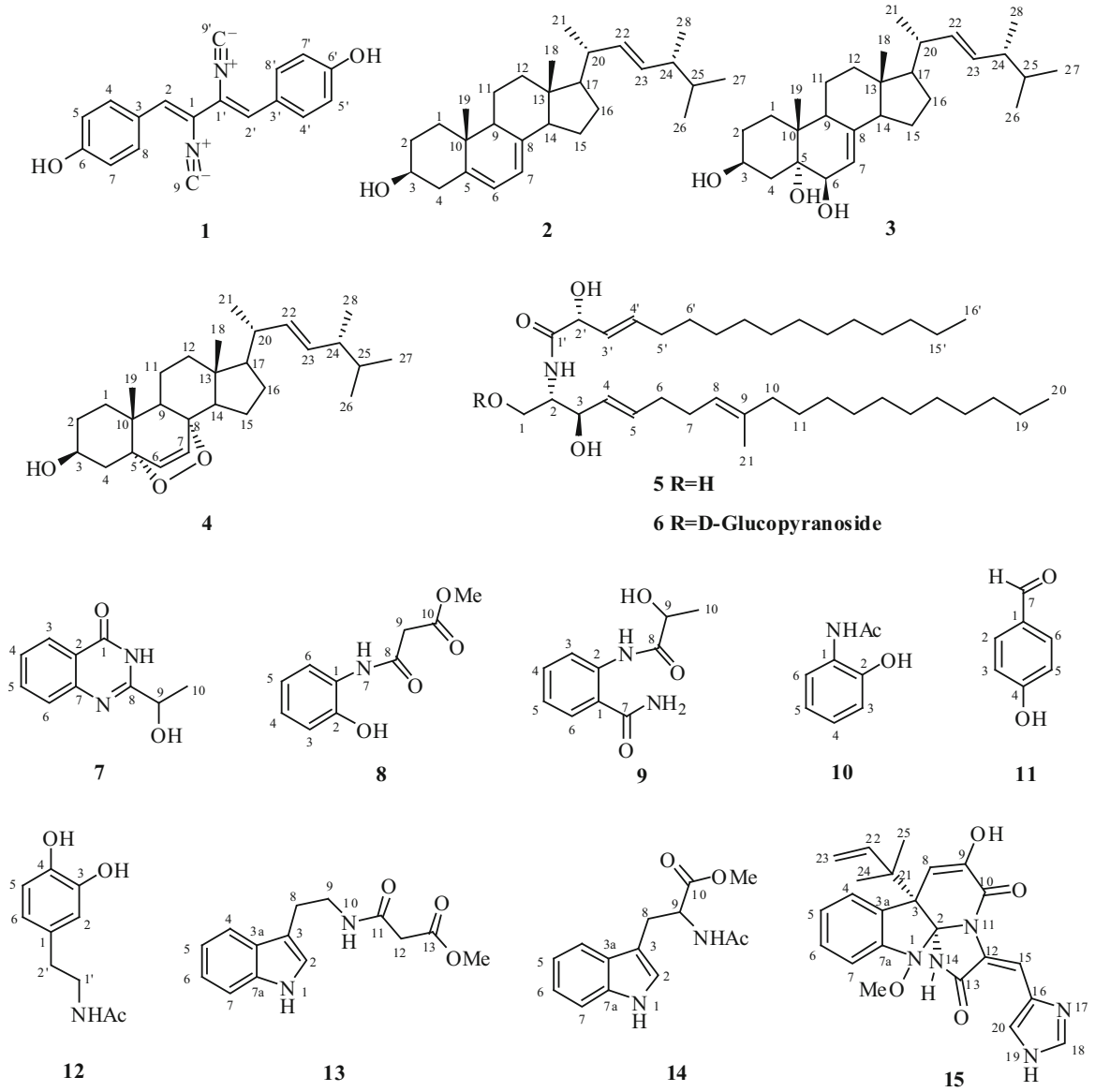

Fig.2 Chemical structure of compounds 1–15

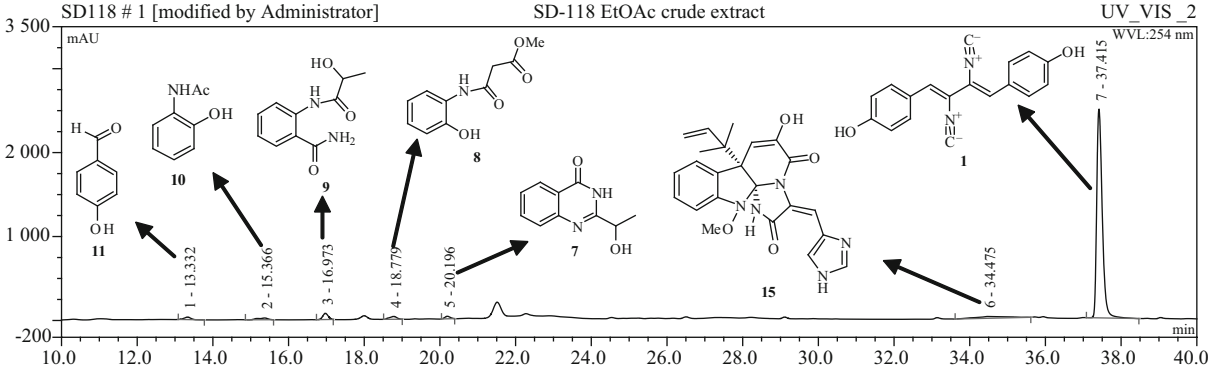

Fig.3 Main compounds identified in the HPLC profile of the *Penicillium commune* SD-118 crude extract

Gram-negative (*E. coli*) microorganisms at 100 μg/disk, with inhibition zone diameters of 16 and 17 mm, respectively. In addition, compound **1** also displayed weak activity toward *A. brassicae* and *F. oxysporium*. The antimicrobial activity of the other compounds is listed in Table 1. The compounds that showed antimicrobial activity were studied further to evaluate the minimum inhibition concentrations (MIC) with broth microdilution method. Compound **1** strongly inhibited the growth of *S. aureus* and *E. coli* with MICs of 2 and 1 μg/mL, respectively, whereas the MICs of the positive control against *S. aureus* and *E. coli* were 1 and 0.5 μg/mL, respectively. In addition, compound **1** showed moderate inhibitory activity against *A. brassicae* with a MIC of 32 μg/mL and compound **15** exhibited weak inhibitory activity toward *S. aureus* with a MIC of 64 μg/mL. The MICs of the remaining compounds were all greater than 256 μg/mL.

Compounds **1**, **7**, and **15** were evaluated for their cytotoxic abilities against MCF-7, SW1990, HepG2, NCI-H460, A549, HeLa, DU145, and MDA-MB-231 cell lines (Table 2). Compound **1** displayed inhibitory activity against the growth of MCF-7, HepG2, NCI-H460, HeLa, DU145, and MDA-MB-231 cell lines with IC_{50} values of 12.0, 7.0, 10.0, 10.0, 8.0, and 8.0 μg/mL, respectively. Among these cell lines, the cytotoxicity of compound **1** toward HepG2 and HeLa cell lines was stronger than that of the positive control fluorouracil (with IC_{50} values of 14 and 14 μg/mL, respectively). To our knowledge, this is the first report of in-vitro cytotoxic activity by xanthocillin X, although several xanthocillin derivatives are strongly toxic toward certain types of tumor cells. Compound **7** only exhibited moderate cytotoxicity activity against the SW1990 cell line with an IC_{50} value of 20.0 μg/mL, whereas compound **15** showed potent cytotoxicity activity toward the DU145 cell line with an IC_{50} value of 5.0 μg/mL, and moderate cytotoxicity toward the HepG2, NCI-H460, HeLa, and MDA-MB-231 cell lines with IC_{50} values of 12.0, 22.0, 20.0, and 11.0 μg/mL, respectively.

In summary, we described the secondary metabolites of the deep-sea sediment-derived fungus *Penicillium commune*. As the main secondary metabolite of this species, the bioactive compound xanthocillin X (**1**) was isolated by bioassay-guided fractionation from a marine-derived fungus for the first time. Compound **1** exhibited significant inhibitory activity against both *S. aureus* and *E. coli*, as well as six tumor cell lines (MCF-7, HepG2, NCI-H460, HeLa, DU145, and MDA-MB-231 cell lines). Compound **15** also showed potent cytotoxicity activity toward the DU145 cell line.

Table 1 Antimicrobial activity of compounds 1–15 against two bacterial and five fungal species. The data represent the diameter of the inhibition zone (mm)

Compound[a]	Test microorganisms and the diameters of inhibition zones (mm)						
	S. aureus	*E. coli*	*A. brassicae*	*F. oxysporium*	*C. diplodiella*	*P. piricola*	*A. niger*
1	**16**	**17**	**8**	**7**	-	-	-
11	-	**7**	-	-	-	-	-
12	-	-	-	**6**	-	-	-
15	**6**	-	-	-	-	**7**	-
Positive control[b]	**22**	**25**	**17**	**9**	**24**	**10**	**19**

a Compounds that were inactive against all tested microorganisms are not listed.
b Chloramphenicol and amphotericin B were used as antibacterial and antifungal positive controls, respectively, at the concentration of 20 μg/disk.

Table 2 Cytotoxic activity of compounds 1, 7 and 15 against eight tumor cell lines. The data represent the IC_{50} (μg/mL)

Compound	Tested tumor cell lines and IC_{50} (μg/mL)							
	MCF-7	SW1990	HepG2	NCI-H460	A549	HeLa	DU145	MDA-MB-231
1	**12**	-	**7**	**10**	-	**10**	**8**	**8**
7	-	**20**	-	-	-	-	-	-
15	-	-	**12**	**22**	-	**20**	**5**	**11**
Fluorouracil[a]	**4**	**16**	**14**	**1**	**7**	**14**	**0.4**	**8**

a Fluorouracil was used as a positive control.

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