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Original Article

Cytotoxic and Antimicrobial Secondary Metabolites from *Penicillium hetheringtonii* IMBC-NMTP04

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Abstract: Chemical investigation of the sesame-associated fungal strain *Penicillium hetheringtonii* IMBC-NMTP04 resulted in isolation of five compounds, penicitrinone A (**1**), dihydrocitrinin (**2**), janthinone (**3**), coniochaetone B (**4**), and 9-oxo-10(*E*),12(*E*)-octadecadienoic acid (**5**). Their chemical structures were elucidated by spectroscopic methods, including 1D and 2D NMR and mass spectra in comparison with the literature data. Compound **1** showed modest cytotoxicity toward LNCaP, HepG2, MCF-7, KB, SK-Mel-2, and HL-60 human cancer cell lines, with IC_{50} values ranging from 60.1 to 84.2 μM while **5** was cytotoxic toward only HL-60 cell line (IC_{50} = 71.6 μM). All the compounds significantly inhibited *E. faecalis*, *S. enterica*, and *C. albicans* growth, with MIC values ranging from 12.5 to 50.0 μM. Compounds **2**, **4**, and **5** suppressed the growth of *B. cereus*, while both **1** and **2** showed antimicrobial effect against *E. coli*, with the same MIC value of 200 μM. This is the first time to report the chemical constituents of *Penicillium hetheringtonii* and the antimicrobial effect of **2**–**4**.

Keywords: Penicillium hetheringtonii, secondary metabolites, cytotoxic; antimicrobial.

1. Introduction

Penicillium is a diverse fungal genus that comprises more than 300 species. The *Penicillium* species are found in various types of substrates, such as soil, food, and in different processes from necrotrophic pathogenicity to endophytic mutualism. Among the *Penicillium* species, *Penicillium hetheringtonii* is a relative new fungal species as it was first isolated from beach soil in 2010 [1]. This fungus is considered to have a close relationship with

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P. citrinum, with several slight differences in morphology, such as having slightly broader stipes, metulae in verticils of four or more [1]. Chemical profile of *P. hetheringtonii* remains little known so far, except that citrinin, quinolactacin, and PR1-x have been reported as its extrolites by using a combination of partial β-tubulin, calmodulin and ITS sequence data, metabolite patterns, and phenotypic characters [1]. In the present study, we report isolation and structural elucidation of five compounds from a fermentation culture of *P. hetheringtonii* IMBC-NMTP-04. Furthermore, cytotoxic and antimicrobial effects of the isolates were also evaluated.

2. Methodology

2.1. Fungal Material

The fungal strain IMBC-NMTP04 was isolated from mouldy sesame collected from a market in Hanoi, Vietnam in 2019. The fungus was taxonomically identified based on the DNA amplification and analysis of the ITS region of the rDNA sequence. By searching the ITS rDNA sequence in comparison with those of the reported fungal sequences in Genbank (website: https://www.ncbi.nlm.nih.gov/genbank/), the fungal strain IMBC-NMTP04 was identified as *Penicillium hetheringtonii* (Genbank accession number: OR288524).

2.2. General Experimental Procedures

Optical rotations were determined using a Jasco P-2000 digital polarimeter. The NMR spectra were recorded on Bruker AVANCE III HD 500 FT-NMR spectrometer. HRESIMS data were obtained using an ESI Q-TOF MS/MS system (AB SCIEX Triple). TLC was performed on Kieselgel 60 F_{254} (Merck) or RP-18 F_{254s} (Merck) plates. Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and YMC RP-18 resins. Preparative high-performance liquid chromatography (HPLC) was performed on an Agilent 1200 Preparative HPLC System.

2.3. Fermentation and Extraction

The fungus *Penicillium hetheringtonii* IMBC-NMTP04 was grown on PDA medium in 100 replicate 2L-Erlenmeyer flasks at the room temperature under static condition for 30 days. The fungal mass growth was then extracted exhaustively with EtOAc under ultrasonic condition to provide an organic phase which was subsequently concentrated under reduced pressure to give a residue (10.56 g).

2.4. Isolation and Identification

The EtOAc extract of *P. hetheringtonii* IMBC-NMTP04 was subjected to a fractionation by reversed phase (RP) C_{18} flash column chromatography (CC), eluting with increasing ratio of MeOH in H_2O to yield six fractions (V1−V6). V2 was subjected to Sephadex LH-20, eluting with MeOH-H_2O (3:1, v/v) to give subfractions V2.1–V2.4. V2.2 was further purified by RP C_{18} prep. HPLC, using an isocratic elution of ACN-H_2O (30:70, v/v) to obtain **2** (7 mg). V3 was fractionated over silica gel, using *n*-hexane-EtOAc (2:1, v/v) as mobile phase to provide three subfractions (V3.1–V3.3). V3.2 was separated by RP C_{18} prep. HPLC, using 30% ACN in H_2O as eluent to furnish **4** (2 mg). V4 was subjected to fractionation using Sephadex LH-20 CC, eluting with MeOH-H_2O (3:1, v/v) to give three subfractions (V4.1–V4.3). V4.1 was separated by silica gel CC, eluting with *n*-hexane-EtOAc (1:1, v/v) and then purified by RP C_{18} prep. HPLC, using 60% ACN in H_2O as eluent to afford **1** (6 mg). V4.2 was separated by RP C_{18} CC, utilizing MeOH-H_2O (5:1, v/v) to provide subfractions V4.2.1 and V4.2.2. V4.2.1 was introduced to silica gel CC, eluting with *n*-hexane-EtOAc (2:1, v/v), and further purified by RP C_{18} prep. HPLC, using ACN-H_2O (55:45, v/v) as mobile phase to give **3** (2.8 mg). Using the similar method, compound **5** (2.1 mg) was isolated from V4.2.2 utilizing RP C_{18} prep. HPLC and ACN-H_2O (60:40, v/v) as mobile phase.

2.5. Cytotoxic Assay

Cytotoxic effects of compounds **1–5** on five human cancer cell lines, including LNCaP,

HepG2, MCF7, KB, SK-Mel-2, and HL-60 were evaluated by SRB assay [2, 3].

2.6. Antimicrobial Assay

Antimicrobial effects of compounds **1–5** toward the Gram-positive bacterial strains *E. faecalis* (ATCC13124), *S. aureus* (ATCC25923), and *B. cereus* (ATCC13245), Gram-negative bacterial strains *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), and *S. enterica* (ATCC12228), and a yeast strain *C. albicans* (ATCC1023) were tested using a micro broth dilution method in 96-well microplates [4].

3. Results and Discussion

*3.1. Spectroscopic Data of Compounds **1–5***

Penicitrinone A (1): white, amorphous powder; $C_{23}H_{24}O_5$; ESIMS: *m/z* 381 $[M+H]^+$; ^{1}H (CD_3OD, 600 MHz): δ_H 5.22 (q, *J* = 6.0 Hz, H-3), 3.33 (m, H-4), 6.30 (s, H-7), 1.44 (d, *J* = 6.6 Hz, H_3-9), 1.36 (d, *J* = 6.6 Hz, H_3-10), 2.13 (s, H_3-11), 4.65 (m, H-2′), 3.28 (m, H-3′), 1.41 (d, *J* = 6.6 Hz, H_3-8′), 1.37 (d, *J* = 7.2 Hz, H_3-9′), 2.25 (s, H_3-10′); ^{13}C NMR data (CD_3OD, 150 MHz): δ_C 161.3 (C-1), 84.4 (C-3), 36.0 (C-4), 134.8 (C-4a), 131.5 (C-5), 185.7 (C-6), 102.8 (C-7), 160.4 (C-8), 100.7 (C-8a), 18.7 (C-9), 19.3 (C-10), 10.8 (C-11), 89.3 (C-2′), 45.9 (C-3′), 142.3 (C-3a′), 118.8 (C-4′), 149.5 (C-5′), 103.8 (C-6′), 137.4 (C-7′), 139.0 (C-7′a), 21.1 (C-8′), 19.4 (C-9′), 11.8 (C-10′).

Dihydrocitrinin (2): white, amorphous powder; $C_{13}H_{16}O_5$; ESIMS: m/z 253 $[M+H]^+$; ^{1}H (DMSO-d_6, 600 MHz): δ_H 4.47 (d, *J* = 15.0 Hz, H_a-1), 4.41 (d, *J* = 15.0 Hz, H_b-1), 3.77 (m, H-3), 2.53 (m, H-4), 1.13 (each d, *J* = 6.6 Hz, H_3-9 and H_3-10), 1.93 (s, H-11); ^{13}C NMR data (CD_3OD, 150 MHz): δ_C 58.7 (C-1), 73.4 (C-3), 34.7 (C-4), 139.3 (C-4a), 109.4 (C-5), 158.2 (C-6), 101.4 (C-7), 155.2 (C-8), 108.3 (C-8a), 20.2 (C-9), 17.9 (C-10), 9.7 (C-11), 175.6 (7-COOH).

Janthinone (3): white, amorphous powder; $C_{16}H_{12}O_5$; ESIMS: *m/z* 285 $[M+H]^+$; ^{1}H (DMSO-d_6, 600 MHz): δ_H 6.72 (s, H-2), 6.96 (s, H-4), 7.78 (d, *J* = 8.5 Hz, H-5), 7.95 (t, *J* = 8.5 Hz, H-6), 7.47 (d, *J* = 8.5 Hz, H-7), 2.43 (s, 3-CH_3), 3.91 (s, 8-OCH_3); ^{13}C NMR data (DMSO-d_6, 150 MHz): δ_C 160.4 (C-1), 111.3 (C-2), 149.7 (C-3), 107.6 (C-4), 155.3 (C-4a), 119.6 (C-5), 136.0 (C-6), 122.8 (C-7), 168.1 (C-8), 116.5 (C-8a), 180.0 (C-9), 106.4 (C-9a), 155.5 (C-10), 22.0 (s, 3-CH_3), 52.7 (s, 8-OCH_3).

Coniochaetone B (4): white, amorphous powder; $C_{13}H_{12}O_4$; ESIMS: m/z 233 $[M+H]^+$; ^{1}H (CD_3OD, 600 MHz): δ_H 5.31 (d, *J* = 6.6 Hz, H-1), 3.18 (m, H_a-2), 2.86 (m, H_b-2), 2.49 (m, H_a-3), 1.99 (m, H_b-3), 6.85 (s, H-7), 6.65 (s, H-9), 2.42 (s, 8-CH_3); ^{13}C NMR data (CD_3OD, 150 MHz): δ_C 71.5 (C-1), 30.6 (C-2), 31.5 (C-3), 175.0 (C-4), 159.1 (C-6), 108.9 (C-7), 148.5 (C-8), 113.3 (C-9), 162.1 (C-10), 110.0 (C-11), 182.4 (C-12), 122.2 (C-13), 22.2 (8-CH_3).

9-oxo-10(E),12(E)-octadecadienoic acid (5): white, amorphous powder; $C_{18}H_{30}O_3$; ESIMS: *m/z* 295 $[M+H]^+$; ^{1}H (CD_3OD, 600 MHz): δ_H 2.29 (br s, H-2), 1.63 (m, H-3, H-7), 1.34 (m, H-4–H-6, H-16, H-17), 2.61 (t, *J* = 7.8 Hz, H-8), 6.15 (d, *J* = 15.6 Hz, H-10), 7.26 (dd, *J* = 9.6, 15.6 Hz, H-11), 6.29 (m, H-12), 6.28 (m, H-13), 2.23 (q, *J* = 6.6 Hz, H-14), 1.49 (m, H-15), 0.93 (t, *J* = 7.2 Hz, H-18); ^{13}C NMR (CD_3OD, 150 MHz): δ_C 178.0 (C-1), 35.3 (C-2), 26.1 (C-3), 30.1 (C-4), 30.2 (C-5 and C-6), 25.6 (C-7), 41.0 (C-8), 204.0 (C-9), 128.8 (C-10), 145.3 (C-11), 130.3 (C-12), 147.4 (C-13), 34.1 (C-14), 29.5 (C-15), 32.5 (C-16), 23.5 (C-17), 14.3 (C-18).

*3.2. Structural Elucidation of Compounds **1–5***

Compound **1** was isolated as a white, amorphous powder. The ^{1}H NMR spectrum exhibited signals for one olefinic proton at 6.30 (s, H-7), two tertiary methyls at 2.13 and 2.25 (each s, H-11 and H-10′) and four secondary methyls at δ_H 1.44, 1.36, and 1.41 (each d, *J* = 6.6 Hz, H-9, H-10, H-8′), and 1.37 (d, *J* = 7.2 Hz, H-9′). Analysis of the ^{13}C NMR and HSQC spectra revealed the occurrence of 23 carbon signals, including one carbonyl carbon at δ_C

185.7 (C-6), 12 sp^2 nonprotonated carbons [of which five are oxygenated at δ_C 161.3 (C-1), 160.4 (C-8), 149.5 (C-5′), 137.4 (C-7′), and 139.0 (C-7′a)], two oxymethines at δ_C 84.4 (C-3) and 89.3 (C-2′), two methines at δ_C 36.0 (C-4) and 45.9 (C-3′), and six methyl carbons. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of the reported citrinin derivative, penicitrinone A revealed the good agreement, suggesting that both structures are identical [5]. In the HMBC spectrum, correlations from H_3-9 to C-3 and C-4, H_3-10 to C-3, C-4, and C-4a, from H_3-11 to C-4a, C-5, and C-6, and from H-7 to C-5, C-6, C-8, and C-8a, from H_3-10′ to C-3′a, C4′, and C-5′ confirmed the presence of a 3,4,5-trimethyl-3,4-dihydro-6*H*-isochromen-6-one structural moiety of **1** (Figure 2). The remaining structure part, 2,3,4-trimethyl-2,3-dihydrobenzofuran-5,7-diol was recognized by HMBC cross-peaks from H_3-10′ to C-3′a, C-4′, and C-5′, from H_3-8′ to C-2′ and C-3′, and from H_3-9′ to C-2′, C-3′, and C-3′a. On the basis of the spectroscopic evidence, compound **1** was identified as penicitrinone A, a metabolite was first isolated from *Penicillium citrinum* [5].

Compound **2** was afforded as a white, amorphous powder. The ^{1}H NMR spectrum contained signals for one oxymethylene [δ_H 4.47 and 4.41 (each d, J = 15.0 Hz, H_a-1 and H_b-1)], one oxymethine at δ_H 3.77 (m, H-3), two secondary methyls [δ_H 1.13 (each d, J = 6.6 Hz, H_3-9 and H_3-10)] and one tertiary methyl at δ_H 1.93 (s, H_3-11). The ^{13}C NMR and HSQC spectra revealed 13 carbon signals, including one carbonyl carbon at δ_C 175.6 (7-COOH), six aromatic nonprotonated carbons [δ_C 139.3 (C-4a), 109.4 (C-5), 158.2 (C-6), 101.4 (C-7), 155.2 (C-8), 108.3 (C-8a)], one oxymethylene at δ_C 58.7 (C-1), one oxymethine at δ_C 73.4 (C-3), one methine at δ_C 34.7 (C-4), and three methyls. This data suggested that **2** belongs to the isochromane skeleton type, which was further supported by an agreement on comparing the ^{1}H and ^{13}C NMR data of **2** with those of the reported isochromane [6]. Finally, the structure of **2** was confirmed by HMBC correlations as shown in Figure 2. Thus, **2** was assigned as dihydrocitrinin.

Compound **3** was isolated as a white, amorphous powder. The ^{1}H NMR spectrum showed typical aromatic signals for AX [δ_H 6.72 and 6.96 (each s, H-2 and H-4)] and ABC [δ_H 7.47 and 7.78 (each d, J = 8.5, H-5 and H-7) and 7.95 (t, J = 8.5, H-6)] spin patterns, one tertiary methyl at δ_H 2.43 (s, 3-CH_3), and one methoxy group at δ_H 3.91 (s, 8-OCH_3). Analysis of ^{13}C NMR and HSQC spectra indicated 16 carbon signals, including two carbonyl carbons at δ_C 180.0 (C-9) and 155.5 (C-10), along with 12 sp^2 carbons of which three oxygen-bearing nonprotonated carbons [δ_C 160.4 (C-1), 155.3 (C-4a), and 168.1 (C-8)] and four nonprotonated carbons [δ_C 149.7 (C-3), 132.9 (C-5a), 116.5 (C-8a), and 106.4 (C-9a)] were recognized. The shielded chemical shift of the carbonyl carbon resonated at δ_C 155.5 suggested the presence of a lactone functional group. The above spectroscopic evidence suggested that **3** has the dibenzoxepindione structural type [7]. Accordingly, the ^{1}H and ^{13}C NMR data of **3** were shown to be in a good match with those of the reported dibenzoxepindione, janthinone, suggesting that both compounds have the identical structures [7]. This was corroborated by HMBC spectrum. As depicted in Figure 2, correlations observed from δ_H 2.43 to C-2, C-3, and C-4 and from δ_H 3.91 to C-7, C-8, and C-8a approved the locations of a methyl at C-3 and a methoxy at C-8, respectively. Based on the spectroscopic analysis, **3** was determined to be janthinone.

Compound **4** was obtained as a white, amorphous powder. In the ^{1}H NMR spectrum, signals of aromatic protons for an AX spin system were observed at δ_H 6.65 and 6.85 (each s, H-7 and H-9) were observed, suggesting the presence of a 1,3,5,6-tetrasubstituted benzene ring. In addition, proton signals for one oxymethine group at δ_H 5.31 (d, J = 6.6 Hz, H-1) and one methyl group at δ_H 2.42 (s, 8-CH_3) were also recognized in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum displayed 13

carbon signals, of which one carbonyl carbon at δ_C 182.4 (C-12), eight sp^2 carbons (of those four were oxygenated [δ_C 175.0 (C-4), 159.1 (C-6) and 162.1 (C-10)]), and four sp^3 carbons [including one oxymethine at δ_C 71.5 (C-1), two methylenes at δ_C 30.6 (C-2) and 31.5 (C-3), and one methyl at δ_C 22.2 (8-CH_3)] were identified using the HSQC experiment. This spectroscopic data suggested that the structure of **4** composes of a benzopyranone in conjugation with a cyclopentane ring [8].

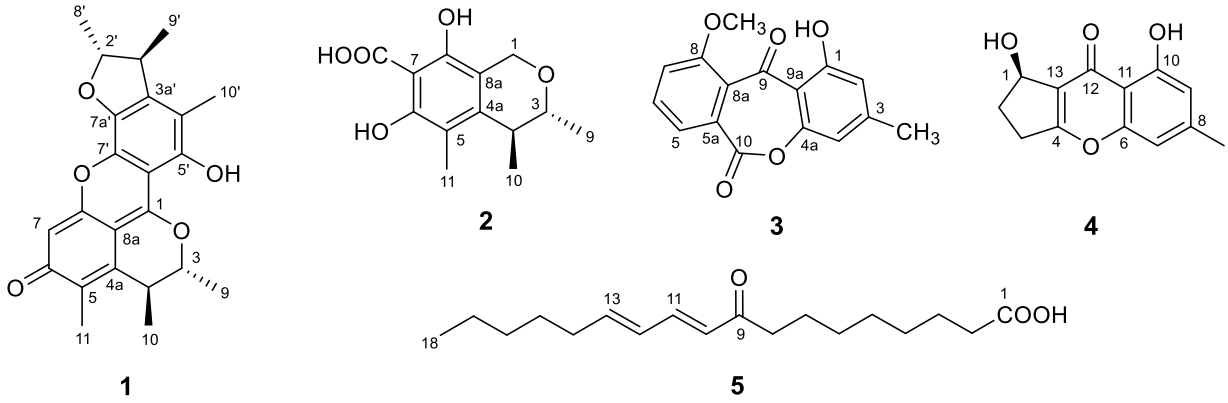

Figure 1. Chemical structures of compounds **1–5** from *P. hetheringtonii* IMBC-NMTP04.

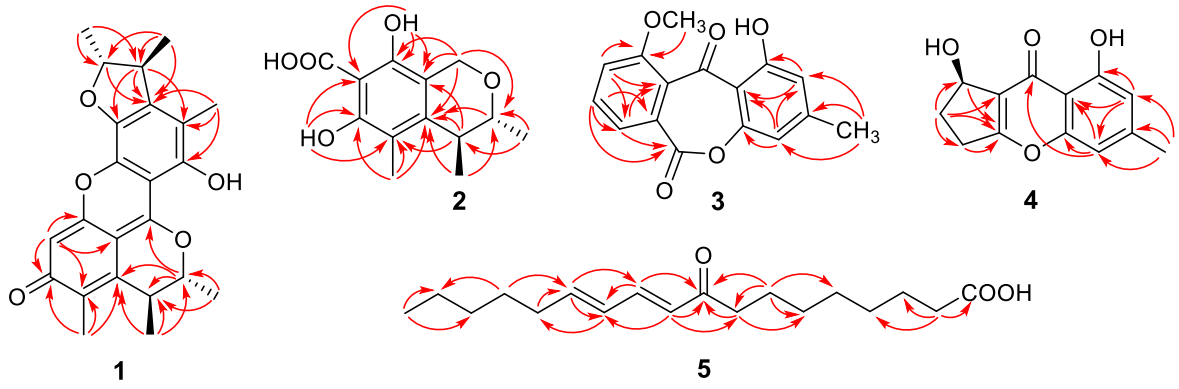

Figure 2. Selected HMBC correlations of compounds **1–5**.

Accordingly, the ^1H and ^{13}C NMR data of **4** were in a good agreement with those of the reported benzopyranone, coniochaetone B, suggesting the similar structures of both compounds. By detailed analysis of the HMBC spectrum, which showed correlations from 8-CH_3 to C-7, C-8, and C-9, from H-7 to C-6 and C-12, and from H_2-2 to C-1, C-3, C-4, and C-12, the planar structure of **4** was established (Figure 2). Based on the aforementioned analysis, together with comparison of the specific optical rotations between **4** ($[\alpha]_D^{28}$ = +38.6 (c = 0.08, MeOH)) and both enantiomers [(+)-coniochaetone B: $[\alpha]_D^{20}$ = +84.70 (c = 0.058, MeOH); (–)-isoconiochaetone B: $[\alpha]_D^{20}$ = –86.96 (c = 0.056, MeOH), compound **4** was elucidated as coniochaetone B [9].

Compound **5** was given as a white, amorphous powder. The ^1H NMR spectrum had signals of four olefinic protons at δ_H 6.15 (d, J = 15.6 Hz, H-10), 7.26 (dd, J = 9.6, 15.6 Hz, H-11), 6.29 and 6.28 (overlapped, H-12 and H-13), implying the presence of two disubstituted double bonds. In addition, a triplet signal of a primary methyl was also observed at δ_H 0.93 (t, J = 7.2 Hz, H_3-18). The ^{13}C NMR

spectrum showed 18 carbon signals, of those one ketone at δ_C 204.0 (C-9), one carbonyl carbon at δ_C 178.0 (C-1), two double bonds [δ_C 128.8 (C-10), 145.3 (C-11), 130.3 (C-12), and 147.4 (C-13)], one primary methyl, and 11 methylene carbons were recognized. Based on this spectroscopic data, **5** was suggested to be an octadecadienoic acid containing two double bonds and one ketone. By comparison of the ^{1}H and ^{13}C NMR data of **5** with a previously reported octadecadienoic acid [10], its structure, 9-oxo-10(*E*),12(*E*)-octadecadienoic acid was established. This was confirmed by HMBC correlations observed from H_2-2 to C-1, C-3, and C-4, from H_3-18 to C-17 and C-16, from H-10 to C-8, C-9, C-12, from H-11 to C-9, C-12, C-13, from H-12 to C-13, C-14, and from H-13 to C-12, C-11, C-14 (Figure 2).

Table 1. Cytotoxic effects of compounds **1–5** at concentration of 100 μM

Compound	Cell death (%)					
	LNCaP	HepG2	MCF-7	SK-Mel-2	KB	HL-60
1	64.5	60.4	67.4	71.2	72.1	89.03
2	14.2	15.3	21.6	15.3	26.9	14.30
3	34.1	23.6	28.8	25.6	47.6	21.45
4	14.6	22.0	13.3	8.4	28.9	48.30
5	18.1	20.0	19.9	35.1	42.5	74.49
Ellipticine*	70.08	71.51	78.93	79.36	82.59	86.8

(*): positive control at concentration of 2 μg/mL.

Table 2. Antimicrobial effects of compounds **1–5**

Compound	MIC values (μM)						
	EF	SA	BC	EC	PA	SE	CA
1	25	-	-	200	-	25	12.5
2	12.5	-	200	200	-	12.5	6.25
3	12.5	-	-	-	-	25	50
4	12.5	-	200	-	-	25	25
5	12.5	-	100	-	-	12.5	12.5
Steptomycin*	256	256	128	32	256	128	-
Nystatin*	-	-	-	-	-	-	8

EF: *Enterococcus faecalis* ATCC299212; SA: *Staphylococcus aureus* ATCC25923; BC: *Bacillus cereus* ATCC13245; EC: *Escherichia coli* ATCC25922; PA: *Pseudomonas aeruginosa* ATCC27853; SE: *Salmonella enterica* ATCC13076; CA: *Candida albicans* ATCC10231; (-): inactive; (*) positive control.

*3.3. Cytotoxic Effect of Compounds **1–5***

Cytotoxicity of compounds **1–5** was evaluated using LNCaP, HepG2, MCF-7, KB, and SK-Mel-2 human cancer cell lines using the sulforhodamine B (SRB) assay at the concentration of 100 μM [2, 3]. The result showed that only penicitrinone A (**1**) displays cytotoxicity toward all the cancer cell lines, with the induction of 60.4–72.1% cell death, whereas **5** induced 74.49% HL-60 cell death (Table 1). Compounds **3** and **5** were cytotoxic against only KB cell line, with induction of 47.6 and 42.5% cell death, respectively while other compounds were considered to be noncytotoxic toward all the cell lines. Based on the preliminary screening results, **1** and **5** were further examined their cytotoxicity at different concentrations. As the result, **1** showed cytotoxicity toward LNCaP, HepG2, MCF-7, KB, SK-Mel-2, and HL-60 cell lines, with IC_{50} values of 77.1 ± 4.1, 84.2 ± 4.7, 74.8 ± 3.9, 71.1 ± 7.5, 60.1 ± 3.9, and 64.0 ± 3.2 μM, respectively, while

5 was cytotoxic toward HL-60 cell line, with an IC_{50} value of 71.63 ± 3.76 μM.

*3.4. Antimicrobial Effect of Compounds **1–5***

Effect of **1**–**5** on the growth of Gram-positive (*Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC13245) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Salmonella enterica* ATCC13076), and a yeast *Candida albicans* ATCC 24433 were also examined [4]. As the result, all the compounds significantly inhibited *E. faecalis*, *S. enterica* and *C. albicans* growth, with MIC values ranging from 12.5 to 50.0 μM (Table 2). In addition, compounds **2**, **4**, and **5** suppressed the growth of *B. cereus*, with MIC values of 200, 200, and 100 μM, respectively, while both compounds **1** and **2** showed antimicrobial effect against *E. coli*, with MIC values of 200 μM.

4. Conclusion

Five compounds, penicitrinone A (**1**), dihydrocitrinin (**2**), janthinone (**3**), coniochaetone B (**4**), and 9-oxo-10(*E*),12(*E*)-octadecadienoic acid (**5**) were isolated from a culture fermentation of the sesame-associated fungal strain *P. hetheringtonii* IMBC-NMTP04. Compound **1** showed modest cytotoxicity toward LNCaP, HepG2, MCF-7, KB, SK-Mel-2, and HL-60 human cancer cell lines while **5** was cytotoxic toward only HL-60 cell line. It is noted that this is the first isolation of 9-oxo-10(*E*),12(*E*)-octadecadienoic acid from the genus *Penicillium* and all compounds from the fungus *P. hetheringtonii*. Furthermore, this is also the first case to communicate the antimicrobial effects of **2**–**4**.

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References

[1] J. A. M. P. Houbraken, J. C. Frisvad, R. A. Samson, Taxonomy of *Penicillium citrinum* and Related Species, Fungal Divers, Vol. 44, No. 1, 2010, pp. 117-133, https://doi.org/10.1007/s13225-010-0047-z.

[2] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines, J. Natl Cancer Inst, Vol. 83, No. 11, 1991, pp. 757-766, https://doi.org/10.1093/jnci/83.11.757.

[3] N. T. T. Ngan, N. H. Hoang, N. T. Hien, N. N. Lan, N. T. K. Lien, T. H. Quang, N. X. Cuong, N. H. Nam, C. V. Minh, Cytotoxic Phenanthrenes and Phenolic Constituents from the Tubers of *Dioscorea persimilis*, Phytochem Lett, Vol. 40, 2020, pp. 139-143, https://doi.org/10.1016/j.phytol.2020.10.005.

[4] T. H. Quang, N. V. Phong, L. N. Anh, T. T. H. Hanh, N. X. Cuong, N. T. T. Ngan, N. Q. Trung, N. H. Nam, C. V. Minh, Secondary Metabolites from a Peanut-Associated Fungus *Aspergillus niger* IMBC-NMTP01 with Cytotoxic, Anti-inflammatory, and Antimicrobial Activities, Nat Prod Res, Vol. 36, No. 5, 2022, pp. 1215-1223, https://doi.org/10.1080/14786419.2020.1868462.

[5] D. Wakana, T. Hosoe, T. Itabashi, K. Okada, G. de Campos Takaki, T. Yaguchi, K. Fukushima, K. I. Kawai, New Citrinin Derivatives Isolated from *Penicillium citrinum*, J. Nat Med, Vol. 60, No. 4, 2006, pp. 279-284, https://doi.org/10.1007/s11418-006-0001-2.

[6] J. Deruiter, J. M. Jacyno, R. A. Davis, H. G. Cutler, Studies on Aldose Reductase Inhibitors from Fungi. I. Citrinin and Related Benzopyran Derivatives, J. Enzyme Inhib, Vol. 6, No. 3, 1992, pp. 201-210, https://doi.org/10.3109/14756369209020170.

[7] Z. Guo, F. Cheng, K. Zou, J. Wang, Z. She, Y. Lin, Secondary Metabolites from the Mangrove Endophytic Fungus *Penicillium* sp. (SBE-8), Nat Prod Commun, Vol. 4, No. 11, 2009, pp. 1934578X0900401108, https://doi.org/10.1177/1934578X0900401108.

[8] H. J. Wang, J. B. Gloer, J. A. Scott, D. Malloch, Coniochaetones A and B: New Antifungal Benzopyranones from the Coprophilous Fungus *Coniochaeta saccardoi*, Tetrahedron Lett, Vol. 36, No. 33, 1995, pp. 5847-5850, https://doi.org/10.1016/0040-4039(95)01174-G.

[9] X. Wei, C. Feng, X. H. Li, X. X. Mao, H. B. Luo, D. M. Zhang, L. Rong, Z. Y. Xie, X. Yu, J. Li, W. C. Ye, X. J. Huang, C. X. Zhang, Enantiomeric Polyketides from the Starfish-Derived Symbiotic Fungus *Penicillium* sp. GGF16-1-2, Chem Biodivers, Vol. 16, No. 6, 2019, pp. e1900052, https://doi.org/10.1002/cbdv.201900052.

[10] M. Yoshikawa, H. Shimada, H. Matsuda, J. Yamahara, N. Murakami, Bioactive Constituents of Chinese Natural Medicines, I. New Sesquiterpene Ketones with Vasorelaxant Efect from Chinese Moxa, the Processed Leaves of *Artemisia argyi* LEVL. et VANT: Moxartenone and Moxartenolide, Chem Pharm Bull, Vol. 44, No. 9, 1996, pp. 1656-1662, https://doi.org/10.1248/cpb.44.1656.