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Original Research Article

Viridicatin and Dehydrocyclopeptine, two bioactive alkaloids isolated from marine derived fungus Penicillium aurantiogriseum AUMC 9759

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Chemical investigation of the marine derived fungus *Penicillium aurantiogriseum* AUMC 9759 isolated from sea sediment of Mediterranean Sea (Alexandria) has led to isolation of two bioactive alkaloids, Viridicatin (1) and Dehydrocyclopeptine (2) along with sub faction B (mixture of two sterols). The compounds were isolated and purified by combined chromatographic procedures.The structures were established by ^{1}H and ^{13}C NMR experiments and HRESIMS data. This is the first report for dehydrocyclopeptine^{1}H and ^{13}C NMR assignments. LC-MS of total fungal extract revealed the presence of cyclopeptine, dehydrocyclopeptine, viridicatin, terrestric acid, viridicatic acid, roquefortine c and 4-hydroy-3,6-Dimethyl-2H-pyran-2-one. The crude extract of the strain exhibited high activities against four bacterial and four fungal strains. Viridicatin (1) showed strong activity against *Mycobacterium tuberculosis*. Sub faction B showed significant cytotoxicity against two cell line; hepatic cellular carcinoma (HEPG2) and breast cancer (mcf7) with IC_{50}% of 32.8774 µg/ml and 24.3284 µg/ml respectively.

Key words: Viridicatin, Dehydrocyclopeptine, cytotoxicity, antimicrobial, *P. aurantiogriseum*

INTRODUCTION

Fungi isolated from marine organisms or marine environment have shown enormous potential as suggested by the diversity of secondary metabolites (Bugni and Ireland, 2004). As a special ecosystem, marine sediment provides an abundant of fungal resources, which yielded various secondary metabolites with novel structures and interesting biological activities (Blunt et al., 2011; Jangala et al., 2013). Marine-derived fungal strains majorly produce polyketide-derived alkaloids, terpenes, peptides, and mixed biosynthesis compounds, which are representative groups of secondary metabolites produced by fungi.Screening members of the genus *Penicillium* for the alkaloids production revealed that most of them produce alkaloids belonging to various structural groups, mainly clavines, diketopiperazines, benzodiazepines and quinolines (El-Shanawany et al., 2005; Xin et al., 2007). In particular, they have a variety of biological activities such as antimicrobial, antitumor, antipredator, antiinflammatory, and antiviral (Zhuravleva et al., 2012).The aim of our study to explore bioactive compounds (antibacterial, antifungal, and antitumor) from *P. aurantiogriseum* AUMC 9759 isolated from sea sediment of Mediterranean Sea and characterization of isolated compounds.

MATERIALS AND METHODS

Fungal isolation and culture conditions

P. aurantiogriseum (Figure 1) was isolated from sea

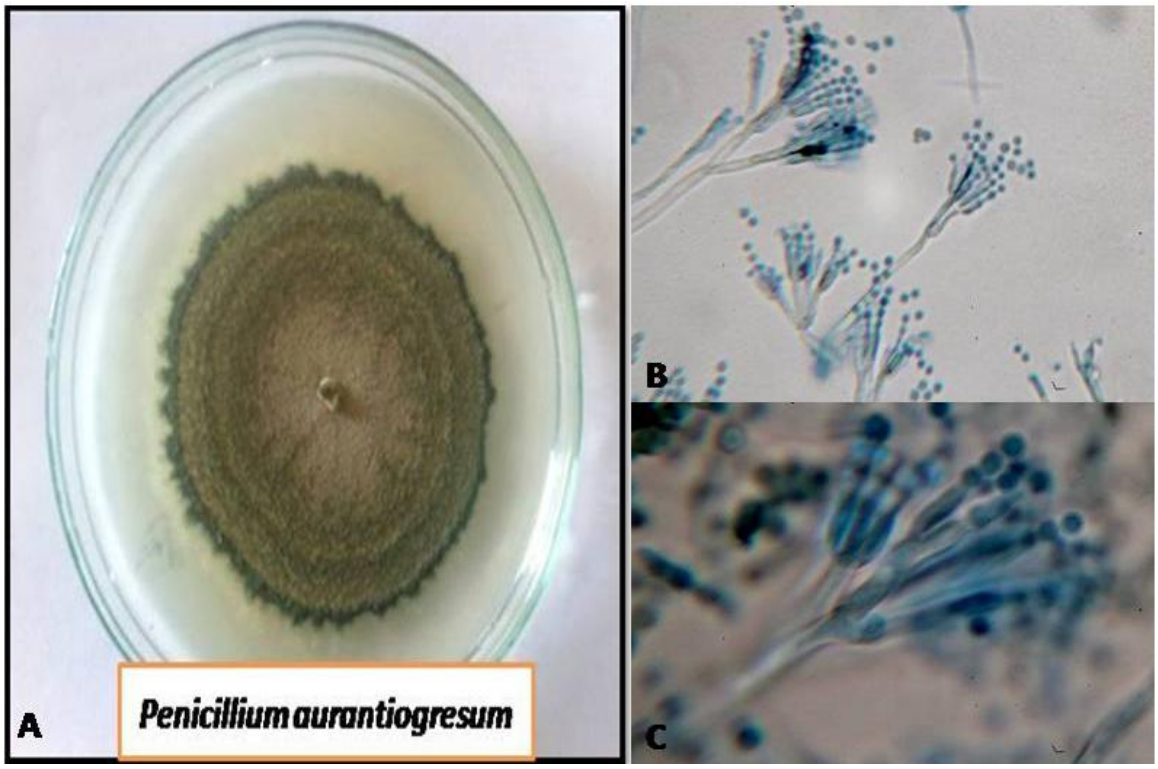

Figure 1: *P. aurantiogresum* AUMC 9759 (A- Cultivated on Czapek-Dox agar medium, B- under light microscope with cotton blue stain X40, C-X 100)

sediment of Mediterranean Sea (Alexandria). The identification was performed by Assuit University Mycology centre (AUMC) based on morphology and ITS sequence analysis and the strain was coded as AUMC 9759 (Pitt and Hocking, 2009). The fungal strain was cultured on rice solid medium at room temperature under static conditions for 15 days, Ninety Erlenmeyer flasks (250 ml) containing 100 gm of rice and 100 ml of distilled water per flask were autoclaved twice at 121°C for 40 minutes. After autoclaving, a small disc of Czapek-Dox agar medium from the Petri dish containing mycelium of selected strain was transferred under sterile conditions to the flasks containing sterilized rice; three flasks were kept for control purposes (Fill et al., 2013).

Extraction of bioactive compounds

After incubation period was finished, the fermentation product was extracted by ethyl acetate. The content of each flask was milled with solvent by using vertical blinder then filtrated by filter paper. The filtrates were collected and evaporated to near drying by using rotary evaporator (Fill et al., 2013).The crude extract was applied to thin layer chromatography (TLC). One hundred micro Letter was spotted 2 cm from the bottom of a precoated aluminum sheet of silica gel 60 F254 (Merk) and running with different mobile system (chloroform: methanol: 25% ammonia 90:10:0.1), (chloroform: methanol 90:10), and (chloroform: methanol 95:5). After running the silica gel sheet allowed to dry after that, the TLC plate was sprayed by Van Urk 's reagent, the plate exposed to heating for few minutes until appear characteristic colors under daylight.

Fractionation of bioactive compounds

The crude extract (10g) was subjected to silica gel column chromatography using *n*-hexane: EtOAc gradients to obtain 5 major subfractions: fraction A (0.9 g), fraction B (1.8 g), fraction C (2.4 g), fraction D (1.7 g) and fraction E (1.7 g). Fraction C was subjected for further purification by solid phase extraction followed by reversed phase-HPLC using a Waters Sunfire RP C18 (C18, 5 µm, 10 mm x 250 mm) column using mobile system MeOH/H2O gradients to afford compound 1 (30 mg) and compound 2 (10 mg). Fraction B subjected to silica gel column chromatography using *n*-hexane: EtOAc gradients to yield sub fraction B (Figure 2).

Identification of bioactive compounds

^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Varian Inova 400 MHz NMR spectrometer. High-resolution mass spectra were acquired with a Thermo scientific LTQ/XL Orbitrap, specifications; analyzer: FTMS, mass range: normal full ms 100-2000, resolution: 30,000. For LC-ESIMS, gradient separation was achieved using a Sun Fire C-18analytical HPLC column (5 mm, 4.6×150 mm, Waters)

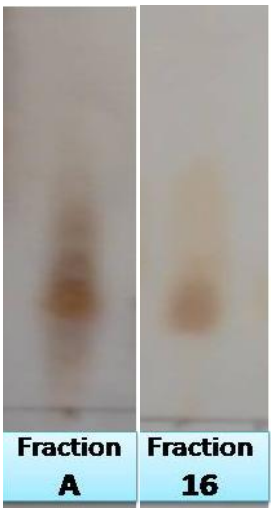

Figure 2: Running fractions (A and 16) on TLC using mobile system (Hexane: Ethyl acetate 8:2)

with a mobile phase of 0-100% MeOH over 30 min at a flow rate of 1 mL/min. HPLC was performed on Waters Sunfire RP C18 (C18, 5 µm ,10 mm × 250 mm) column connected to an Agilent 1200 series binary pump and monitored using an Agilent photodiode array detector. Detection was carried out at 220, 254, 275, and 320 nm. All chemical reagents were purchased from Sigma/Aldrich and used without further purification.

Viridicatin (1)

3-hydroxy-4-phenylquinolin-2(1H)-one.$C_{15}H_{11}NO_2$, pale brown crystalline, HRESIMS m/z 238.09 $(M+H)^+$. ^1H NMR (DMSO-d_6) 12.24 (1H, bs, N–H), 9.21 (1H, bs, O–H), 7.03-7.34 (3H, m interchangeable, H-5 to H-7), 7.50 (1H, d J=8.0 Hz, H-8), 7.31-7.51 (5H, m interchangeable, H-2` to H-6`). ^{13}C NMR (DMSO-d_6) 158.3 (C-2), 142.4 (C-3), 127.6 (C-4), 126.4 (C-5), 122.1 (C-6), 123.9 (C-7), 115.2 (C-8), 120.9 (C-9), 133.1 (C-10), 133.7 (C-1`), 129.8 (C-2`), 128.3 (C-3`), 124.3 (C-4`), 128.3 (C-5`), 129.8 (C-6`).

Dehydrocyclopeptine(2)

(Z)-3-benzylidene-3,4-dihydro-4-methyl-1H-benzo[e][1,4]diazepine-2,5-dione. $C_{17}H_{14}N_2O_2$, white amorphous powder, HRESIMS m/z 279.11$(M+H)^+$. ^1H NMR (CD_3OD-d_4) 12.24 (1H, bs, N–H), 9.21 (1H, bs, O–H), 7.91 (1H, brd*J* = 8.0 Hz, H-6), 7.28 (1H, brt*J* = 7.50 Hz, H-7), 7.54 (1H, brt*J* = 7.50 Hz, H-8), 7.13 (1H, brd*J* = 8.0 Hz, H-9), 6.92 (1H, s, H-12), 7.35 (2H, d, *J* = 8.0 Hz, H-2`/H-6`), 7.39 (2H, d, *J* = 8.0 Hz, H-3`/H-5`), 7.42 (1H, t *J* = 7.50 Hz, H-4`), 3.13 (3H, s, N–CH3).$^{13}C$ NMR ($CD_3OD$-$d_4$) 172.3 (C-2), 133.6 (C-3), 169.0 (C-5), 135.3 (C-6), 131.8 (C-7), 131.8 (C-8), 125.8 (C-9), 137.9 (C-10), 126.7 (C-11), 122.0 (C-12), 135.3 (C-1`), 130.2 (C-2`), 130.1 (C-3`), 130.9 (C-4`), 130.1 (C-5`), 130.2 (C-6`), 36.1 (N-CH3).

Antimicrobial assay

Screening for antibacterial activity

The antibacterial activity of the marine-derived fungus, *P. aurantiogriseum* crude extract was tested against four different pathogenic bacterial strains. Three of them are Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *B. subtillus*) and one Gram-negative (*Salmonella* sp.). Aliquots of the tested bacterial cultures were introduced into sterile Petri-dishes then poured with sterilized nutrient agar medium then allowed to solidify; filter paper discs were saturated with crude extract and placed on the surface of agar medium. All plates were incubated at 37°C for 24 h, and were observed for the formation of inhibition zones around the discs.

Screening for antifungal activity

The antifungal activity of the marine-derived fungus (crude extract) was tested against four different fungal strains, which belong to *Aspergillus ochraceus*, *A. flavus*, *Fusarium solani*, and *Alternaria alternata*. Aliquots of the tested fungal cultures were introduced into sterilized Petri-dishes then poured with sterilized Czapek's medium and then allowed to solidify. Wells of 0.5 cm were made in the medium by sterilized cork borer, and 150 µl of crude extract were transferred into each well. All plates were incubated at 28°C for 3-5 days, and were observed for the formation of inhibition zones around the wells (Ely et al., 2004).

Determination of antibacterial activity

The purified compounds (compounds 1, 2, and sub fraction B) were tested for their antibacterial activity against six different pathogenic bacterial strains namely: *Mycobacterium tuberculosis, Klebsiella pneumonia*, *Staph. aureus*, *Pseudomonas aeruginosa*, *E. coli* and *proteus vulgaris*by disc diffusion method.

Determination of antitumor activity

The purified compounds have been tested for their antitumor activity against two cell line hepatic cellular carcinoma (HEPG2) and breast cancer (mcf7) by SRB (sulforhodamine B) assay

Cell seeding for sulforhodamine B (SRB) assay (original monolayer 96-well plate seeding)

Potential cytotoxicity of the compounds were tested using the method of Skehan et al. (1990). Cells under investigation were trypsinized and proper dilution in the compatible media was made. Aliquots of 100 µl cell

Table 1. Screening crud extracts of *P. aurantiogriseum* AUMC 9759 cultivated on rice medium against selected fungal and bacterial strains

Fungal and bacterial strains	Ethyl acetate extract
Aspergillus ochraceus	**++ve**
Aspergillus flavus	**+ve**
Fusarium solani	**+++ve**
Alternaria alternate	**++ve**
Staphylococcus aureus	**+++ve**
Bacillus cereus	**++ve**
Bacillus subtillus	**++ve**
Salmonella typhi	**++ve**

(+ve=low activity, ++ve=moderate activity and +++ve=high activity)

suspension containing 1000 cells were seeded into flat bottom 96-well plate (according to the cell line doubling time, and operator handling usually ranges from 500-2000 cell per well). Negative control: a lane that only contains media, while positive control is a lane that contains cells but not treated. Each plate must accommodate at least 24 peripheral wells filled with PBS; to guard against media drying. Plates were incubated in a humidified 37°C, 5% CO2 chamber for 24 hr. Another aliquots of 100µl media containing the compounds concentration ranging from 1 µg/ml to 100 µg/ml were added to treated lanes, and blank media to the +ve, and –ve control lanes (this is considered the zero time for treatment). Plates were incubated in a humidified 37°C, 5% CO2 chamber for another 72 hrs (for the long incubation period the media might need to be changed, and some time PBS washing is recommended according to the treatment under investigation.

SRB (sulforhodamine B) assay procedure (for simple 96-well plate format)

On the day of analysis, the 96 well-plates were centrifuged at 1000 rpm, and 4 °C for 5 min. The media containing the crude extract solution were removed. Fixation was performed by 150 µl of 10% TCA, added and incubated at 4 ºC for 1 hr. The fixative solution was removed and washed 5 times with double distilled water. Aliquots of 70 µl of 0.4 % SRB solution were added and incubated for 10 min at room temperature in a dark place. Plates were washed 3 times with 1% acetic acid, and left to air-dry overnight. To dissolve SRB-bound protein, aliquots of 150 µl of 10 mMTris- HCl were added and shaken for 2 min. The absorbance was measured at 540 nm (Skehan et al. 1990).

RESULTS AND DISCUSSION

Abo-Kadoum et al. (2013) reported that the screening of fungal strains for indole alkaloids production revealed that *P. aurantiogriseum* has been provide a rich source of mixture of indole alkaloids and exhibited many blue spots with Van Urk's reagent on TLC. As part of our efforts toward the chemical investigation of marine-derived fungi, a variety of structurally interesting and biologically active compounds were isolated and identified from *P. aurantiogriseum* AUMC 9759.The ethyl acetate crude extract of *P. aurantiogriseum* cultured on solid rice medium exhibited high significant activity against *Staphylococcus aureus* and *Fusarium solani*; moderate activity against *Bacillus cereus*, *B. subtillus*, *Salmonella sp*., *Alternaria alternata*, and *A. ochraceus*; and low activity against *Aspergillus flavus* (Table 1). Similarly, Khaddor et al. (2007) reported that *B. subtilis* was the most sensitive to *P. aurantiogriseum* culture filtrate, and *S. typhimurium* was slightly sensitive while *E. coli*, *B. megaterium*, and *Lactococcus diacetylactis* were insensitive to the filtrate. In contrast, Li et al. (2013) reported that no obvious antimicrobial activity observed for five new anthranilic acid derivatives, penipacids A–E, together with one known analogue isolated from the marine sediment-derived fungus *Penicillium paneum* SD-44 when they tested against two bacteria (*Staphylococcus aureus* and *Escherichia coli*) and three plant-pathogenic fungi (*Alternaria brassicae*, *Fusarium graminearum*, and *Rhizoctonia cerealis*).

Interestingly, our study for the first time revealed the biological activity of crude extract of *P. aurantiogriseum* against four mycotoxigenic fungi. MICs less than 10 µg/mL against tested microbes.

Identification and bioassay of purified compounds

Two compounds 1, 2 were purified from fraction A by reversed-phase HPLC (Figures 3), while sub Fraction B was semi purified from fraction B by column chromatography using mobile system (95:5 Hexane: EtOAc). The identification based on data of high-resolution mass spectrum (HRESIMS) and nuclear magnetic resonance (NMR) spectrum (^{1}H and ^{13}C).

Compound 1 was obtained as pale brown crystalline. It has a molecular formula $C_{15}H_{11}NO_2$ (Figures 4) which was determined by HRESIMS quasi-molecular ion peak at m/z 238.09 $(M+H)^+$. ^{13}C NMR revealed the presence of fifteen carbon peaks including 9 aromatic methane speaks resonating between the chemical shift δ_C 115.29 - 129.86. It

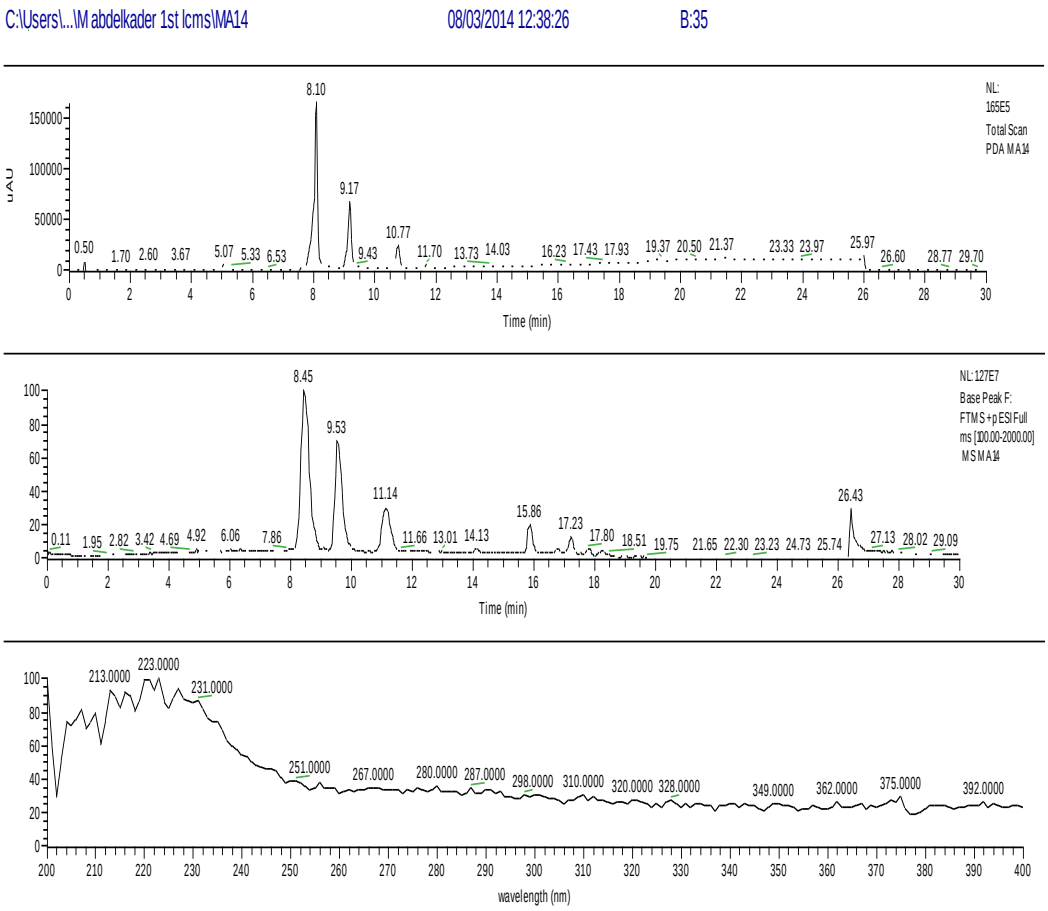

Figure 3: Liquid chromatography- Mass spectrum of fraction A

also showed 6 quaternary carbons, one of them was attributed to amide carbonyl (δ_C158.3, C-2). The ^{1}H NMR spectrum (Table 2) showed nine aromatic protons which were overleaped between δ7.06 and δ7.51.Four protons of them forming close an ABCD system of aromatic ring(H-5 to H-8). In the ^{1}H NMR spectrum there were two signals in downfield region at δ12.24 assigned for one NH group and at δ 9.21 assigned for one hydroxyl group at position 3.Based on these findings, the structure of 1 was unambiguously assigned as viridicatin.

P. aurantiogriseum species have been reported as producers of a wide range of alkaloids with diverse chemical structures. Kozlovsky et al. (2003) found that *P. aurantiogriseum* Dierckx 1901 synthesized the diketopiperazine alkaloids roquefortine and 3,12-dihydroroquefortine. Yu et al. (2010) isolated new secondary metabolites verrucosidinol and its derivative verrucosidinol acetate, together with a potent neurotoxin verrucosidin, a congener norverrucosidin and a mixture of two known phytotoxic metabolites terrestric acids from the marine derived fungus *P. aurantiogriseum*. Song et al. (2012) isolated three new alkaloids, including auranomides A and B, a new scaffold containing quinazolin-4-one substituted with a pyrrolidin-2-iminium moiety, and auranomide C, as well as two known metabolites auranthine and aurantiomides C from the marine-derived fungus *P. aurantiogriseum*. In our study, viridicatin showed strongly antibacterial activity against pathogenic *Mycobacterium tuberculosis*.

Compound 2 was obtained as white amorphous powder. It has a molecular formula $C_{17}H_{14}N_2O_2$(Figures 4) which was determined by HRESIMS quasi-molecular ion peak at m/z279.11(M+H)$^+$. ^{13}C NMR (Table 2) revealed the presence of seventeen carbonpeaksincludinga methylcarbon peak (δ_C36.12), 9aromatic methane carbon peaks(δ_C 125.89—130.94), analiphatic methine carbon peakδ_C122.01, (C-12), and 6 quaternary carbonpeaks;two of them are attributed to amide carbonylcarbonsδ_C172.38(C-2) and δ_C169.04 (C-5).^{1}H NMR spectrum (Table 2) showed singlet methyl signal at downfield shift δ_H 3.31 assigned forN-CH3. Another singlet signal for olefinic proton (δ_H 6.92, H-12) which correlated with olefinic carbons at δ_C 122.01 (C-12). It also showed four aromatic protons(H6-H9) forming common ABCD system. The other aromatic ring protons appear as pair of coupled doublets at δ_H 7.35 and 6.39 (each 2H, J =

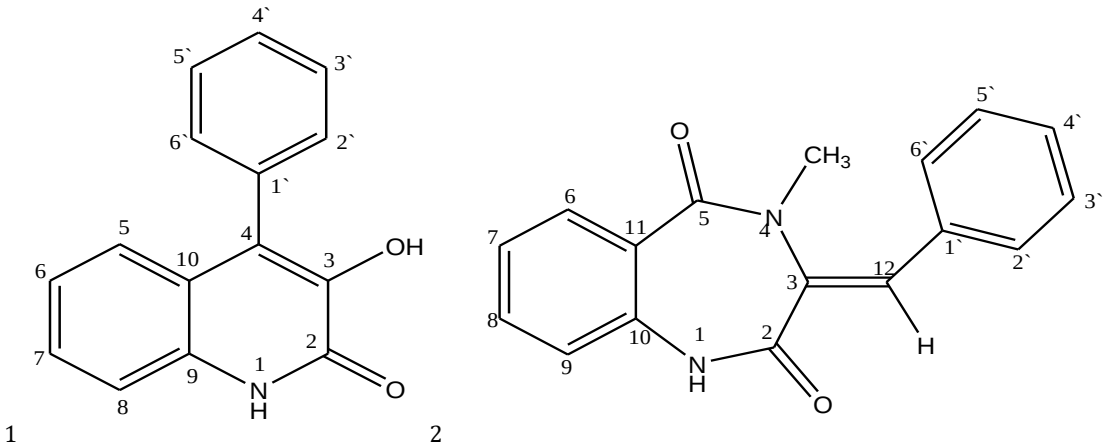

Figure 4: Chemical structures of compounds 1 and 2

Table 2: ^{1}H and ^{13}C NMR spectral data of 1 (DMSO-d_6, 400 and 100 MHz) and 2 (CD3OD-d_4, 400 and 100 M

	1		**2**	
Position	**δ_H [mult., *J* (Hz)]**	**δ_C (mult.)**	**δ_H [mult., *J* (Hz)]**	**δ_C (mult.)**
1	12.24 bs	-	-	-
2	-	158.31 C	-	172.38 C
3	9.21 bs	142.46 C	-	133.60 C
4	-	127.67 C	3.13 s	36.12 CH_3
5	7.03-7.06 m[a]	126.44 CH	-	169.04 C
6	7.31-7.34 m[b]	122.14 CH	7.9 br. d (8.0)	135.33 CH
7	7.03-7.06 m[a]	123.97 CH	7.28 br. t (7.5)	131.82 CH
8	7.50 d (7.8)	115.29 CH	7.54 br.t (7.5)	131.83 CH
9	-	120.93 C	7.13 br. d (8.0)	125.89 CH
10	-	133.18 C	-	137.92 C
11	-	-	-	126.73 C
12	-	-	6.92 s	122.01 CH
1`	-	133.77 C	-	135.33 C
2`	7.31-7.34 m[b]	129.86 CH	7.35 d (8.0)	130.23 CH
3`	7.49-7.51 m[c]	128.35 CH	7.39 d (8.0)	130.17 CH
4`	7.31-7.34 m[b]	124.32 CH	7.42 t (7.5)	130.94 CH
5`	7.49-7.51 m[c]	128.35 CH	7.39 d (8.0)	130.17 CH
6`	7.31-7.34 m[b]	129.86 CH	7.35 d (8.0)	130.23 CH

[abc]chemical shift are interchangeable

8.5 Hz, H-2`/H-6`, H-3`/H-5`) and triplet at δ_H 7.42 (1H, J=7.5 Hz. H-4`.Based on these findings, the structure of **2** was unambiguously assigned as dehydrocyclopeptine**,** which is firstly isolated from this strain.

Sub fraction B may be a mixture of ergosterol derivatives. Several trials to isolate this mixture of bioactive sterols in pure form have failed. In our study, sub fraction B showed different activities against hepatic cellular carcinoma (HEPG2) and breast cancer (MCF-7), with IC50 was 32.8774 and 24.3284 µg/ml respectively (Figures 5, 6). Du et al. (2010) isolated four new alkaloids, including two new meleagrin analogs, meleagrin D and E, and two new diketopiperazines, roquefortine H and I, from a deep ocean sediment-derived fungus *Penicillium sp*. They reported that new meleagrins showed weak cytotoxicity against the A-549 cell line, whereas meleagrin B and meleagrin, which were isolated previously from the same strain, induced HL-60 cell apoptosis or arrested the cell cycle through G(2)/M phase, respectively. Lin et al (2012) isolated penicilliumin A, along with two known compounds ergosterol and ergosterol peroxide from *Penicillium* sp F00120 isolated from a deep sea sediment sample. They reported that penicilliumin A inhibited in vitro proliferation of mouse melanoma (B16), human melanoma (A375), and human cervical carcinoma (Hela) cell lines moderately.

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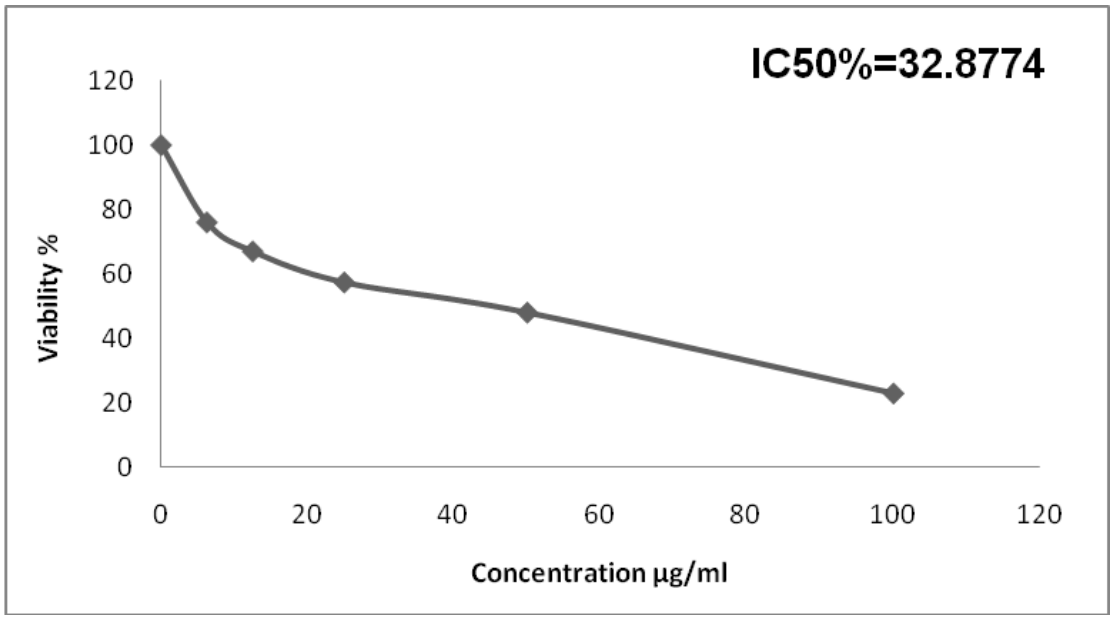

Figure 5: Effect of different concentrations of sub fraction B on viability of cancer liver cells (Hepatic Cellular Carcinoma)

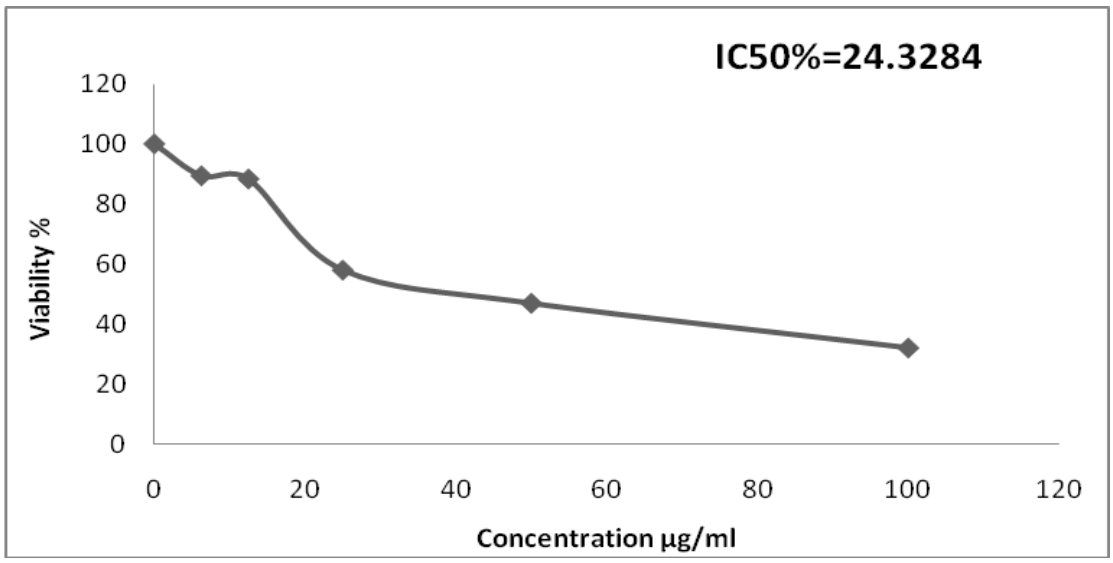

Figure 6: Effect of different concentrations of sub fraction B on viability of cancer breast cells (Michigan Cancer Foundation-7)

Biodiscovery Centre laboratory, University of Aberdeen, UK for allowing running HREIMS and ^{1}H and ^{13}C NMR experiments

REFERENCES

Abo-Kadoum MA, Abo-Dahab NF, Awad MF, Abdel-Hadi AM (2013). Marine-derived fungus, *Penicillium aurantiogriseum* AUMC 9757: a producer of bioactive secondary metabolites. J. Basic Appl. Mycol. 4(1): 77–83.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2011). Marine natural products. Nat. Prod. Rep.28(2): 196-268.

Bugni TS, Ireland CM (2004). Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat.Prod. Rep.21(1):143- 163.

Du L, Feng T, Zhao B., Li D, Cai S, Zhu T, Wang F, Xiao X, Gu Q (2010). Alkaloids from a deep ocean sediment-derived fungus *Penicillium sp*. and their antitumor activities. J. Antibiot. 63 (4): 165-170.

El-Shanawany A, Barakat AA, Abdel-Hadi AM (2005). Ergot

alkaloids agroclavine-1 and epoxyagroclavine-1 from *Penicilliumcitrinum* MU-534. Afric. J. Mycol. Biotech. 13(1): 71-82.

Ely R, Supriya T, Naik CG (2004). Antimicrobial activity of marine organisms collected from the coast of South East India. J. Exper. Mar. Biol. and Ecol. 309 (1): 121-127.

Fill TP, Asenha HBR, Marques AS, Ferreira AG, Rodrigues-Fo E (2013). Time course production of indole alkaloids by an endophytic strain of *Penicilliumbrasilianum* cultivated in rice. Nat. Prod. Res. 27(11): 967–974.

Jangala S, Sowjanya KM, Narendra K, Rathnakar Reddy KVN, Satya AK (2013). Isolation, identification & production of bioactive metabolites from marine fungi collected from coastal area of Andhra Pradesh, India. J. pharm. Res. 6(6): 663-666.

Khaddor M, Saidi R, Aidoun A, Lamarti A, Tantaoui- Elaraki A, Ezziyyani M, Castillo M, Badoc A (2007). Antibacterial effects and toxigenesis of *Penicillium aurantiogriseum* and *P. viridicatum.* Afric. J. Biotech. 6(20) : 2314-2318.

Kozlovsky AG, Zhelifonova VP, Adanin VM, Antipova TV, Ozerskaya SM, Ivanushkina NE, Grafe U (2003). *Penicillium aurantiogriseum* Dierckx 1901: Producer of Diketopiperazine Alkaloids (Roquefortine and 3,12-Dihydroroquefortine), Isolated from Permafrost. Appl. Bioch Microbiol.39(4): 393–397.

Li CS, Li XM, Gao SS, Lu YH, Wang BG (2013). Cytotoxic anthranilic acid derivatives from deep sea sediment-derived fungus *Penicillium paneum* SD-44. Mar. Drugs. 11 (8): 3068-3076.

Lin X, Zhou X, Wang F, Liu K (2012). A new cytotoxic sesquiterpen equinone produced by *Penicillium sp.* F00120 isolated from a deep sea sediment sample. Mar. Drugs. 10 (1): 106-115.

Pitt JI, Hocking AD (2009). Fungi and food spoilage. Springer Dordrecht Heidelberg London New York. pp. 501.

Skehan P, Storeng R, Scudiero D, Monks a, Mcmahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR (1990). New colorimetric cytotoxicity assay for anti cancer drug screening. J. of natl. cancer inst. 82(13): 1107-1117.

Song F, Ren B, Yu K, Chen C, Guo H, Yang N, Gao H, Liu X, Liu M, Tong Y, Dai H, Bai H, Wang J, Zhang L (2012). Quinazolin-4-one coupled with pyrrolidin-2-iminium alkaloids from marine-derived fungus *Penicillium aurantiogriseum*. Mar. Drugs 10(6): 1297-1306.

Xin ZH, Fang Y, Du L, Zhu T, Duan L, Chen J, Gu QQ, Zhu WM(2007). Aurantiomides A–C, quinazoline alkaloids from the sponge-derived fungus *Penicillium aurantiogriseum* SP0-19. J. Nat. Prod.70(5): 853-855.

Yu K, Ren B, Wei J, Chen C, Sun J, Song F, Dai H, Zhang L (2010). Verrucisidinol and verrucosidinol acetate, two pyrone-type polyketides isolated from a marine derived fungus, *Penicillium aurantiogriseum*. Mar. Drugs 8(11): 2744–2754.

Zhuravleva OI, Afiyatullov SS, Denisenko VA, Ermakova SP, Slinkina NS, Dmitrenok PS, Kim NY (2012). Secondary metabolites from a marine-derived fungus *Aspergillus carneus* Blochwitz. Phytoch.80: 123-131.