Fusaproliferin, a fungal phytotoxin shows rapid and potent cytotoxicity against pancreatic cancer cell lines.

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Abstract: As a part of our ongoing research on endophytic fungi, we have isolated a sesterterpene mycotoxin, fusaproliferin (FUS), from *Fusarium solani* strain associated with the plant *Aglaonema hookerianum* Schott. FUS showed rapid and sub-micromolar IC$_{50}$ against pancreatic cancer cell lines. Time dependent survival analysis and microscopy imaging showed rapid morphological changes in cancer cell lines 4 hours after incubation with FUS. This provides a new chemical scaffold that can be further developed to obtain more potent synthetic agents against pancreatic cancer.

Keywords: endophytic fungi; sesterterpene; cytotoxic activity, pancreatic cancer

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1. Introduction

Pancreatic adenocarcinoma is a leading cause of adult cancer mortality. It is presently untreatable, with a 5-year survival rate of ~5% [1]. As early detection is difficult, most patients present with locally advanced or metastatic disease [2]. Therapeutic options are limited and metastatic disease frequently develops after surgery [3]. Pancreatic cancer is the seventh leading cause of cancer-related deaths worldwide and annually more than 200,000 deathsare attributed to pancreatic cancer every year [4,5]. Natural sources, particularly plants, represent an important source of new anticancer chemical scaffolds, and there is an increasing interest in searching for natural products with drug-like properties as potential leads for drug discovery projects [6,7].

Endophytic fungi are symbiotically associated with plants, capable of synthesizing bioactive compounds without causing any damage to the host [8]. Some of these compounds have proven useful for novel drug discovery and also provide a defense against harmful pathogens for the plants [9,10]. As a part of our ongoing research on endophytic fungi [11-14], a *Fusarium solani* strain, isolated from the plant *Aglaonema hookerianum* Schott. (Family: Araceae), was investigated. *Fusarium solani* has been proved as a potent source of structurally diverse natural compounds with cytotoxic activity, such as karuquinone A and karuquinone B [15], 9-desmethylherbarine, 7-desmethylscorpinone and 7-desmethyl-6-methylbostrycoidin [13], camptothecin and 10-hydroxycamptothecin [16], as well as paclitaxel [17]. Chemical investigation of the ethyl acetate extract of the *F. solani* led to the isolation of fusaproliferin (FUS), a mycotoxin which was first isolated from the Italian *F. proliferatum* strains, named as proliferin [18]. FUS is also produced by *Fusarium subglutinans* and fifteen other ex-type strains of *Fusarium* species [19,20]. FUS produced toxic effect on *Artemia salina*, insect cells and human B lymphocytes [21]. It was also reported to produce teratogenic effect on chicken embryos [22]. In this study, we examined the anticancer activity of FUS against two pancreatic and two breast cancer cell lines as well as compared the activity of the compound with that of gemcitabine and doxorubicin, the current drugs of choice for pancreatic and breast cancer, respectively.

2. Results and Discussion

FUS was obtained as white gum. The structure of FUS (Figure 1) was confirmed by spectroscopic analysis (^{1}H, ^{13}C NMR, DEPT-135, 2D-NMR and HR-ESIMS) and by comparison with the published spectral values [18]. Accurate mass measurement of FUS obtained by FT-ESI-MS yielded a parent mass at *m/z* 467.2778 in positive ionization mode, corresponding to the sodium adduct [M +Na]$^+$ with a molecular formula of $C_{27}H_{40}O_5$ (calcd mass 467.2773, [$C_{27}H_{40}O_5$+ Na]$^+$), accounting for 8 degrees of unsaturation. The resonances at δ 170.9 and 207.9 ppm in the ^{13}C NMR spectrum were characteristics for the presence of two carbonyl carbons of an ester and a ketone, respectively. The ^{1}H and ^{13}C NMR data, in conjunction with the DEPT 135 spectrum (Figure S13), proved the presence of 27 carbon atom signals corresponding to six methyls (20-, 21-, 22-, 23-, 25-, and 27-), seven sp^3 methylenes (1-, 4-, 5-, 8-, 9-, 13- and 24-), three sp^3 methines (10-, 14- and 19-), three sp^2 methines (2-, 6- and 12-), one sp^3 quaternary carbon (15-), five sp^2 quaternary carbons (3-, 7-, 11-, 17- and 18-), two carbonyl carbons of an ester (26-OCOCH$_3$) and a ketone (16-CO-). The presence of three sp^2 methines and five sp^2 quaternary carbons, along with one each of an ester and carbonyl moiety, proved the presence of six double bonds in this compound and thus indicated it as a bicyclic compound. After deducting the acetyl moiety 'OCOCH$_3$' (δ_H = 2.06, δ_C = 20.9 and 170.9 ppm), the compound consists of 25 carbons, which indicated it as a sesterterpene.
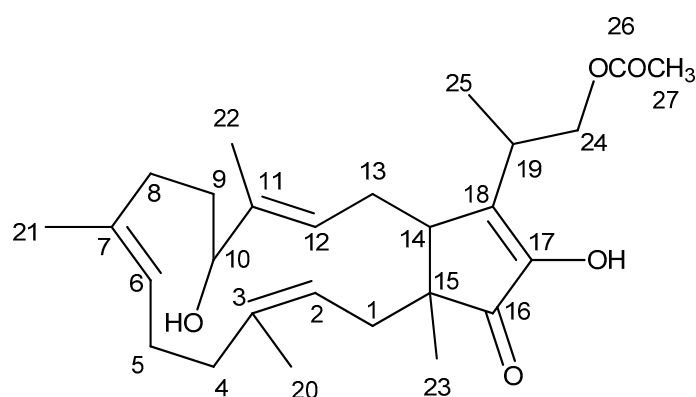

Figure 1. Structure of Fusaproliferin

Table 1. Cytotoxicity assay results of FUS against human tumor cells (IC$_{50}$ in μM)

Compound/ Standard	MIA PaCa 2 (Pancreatic)	BXPC3 (Pancreatic)	MDA MB 231 (Breast)	MCF7 (Breast)
FUS	0.13±0.09	0.76±0.24	1.9±0.32	3.9±0.75
Gemcitabine	7.6±0.66	2.2±0.43	NT	NT
Doxorubicin	NT	NT	0.06±0.03	0.02±0.018

The cytotoxicity of FUS was determined against two pancreatic cancer cell lines BxPc3 and MIA PaCa2 and the ER-positive breast cancer cell line MCF7 and triple negative breast cancer cell line MDA MB 231; gemcitabine was used as the positive control for the pancreatic cancer cell lines and doxorubicin for the breast cancer cell lines. FUS was active against all four cell lines tested (Figure 2 and Table 1) with sub to low micromolar IC_{50}, but the activity against the pancreatic cancer cell lines were notably better than the breast cancer cell lines. FUS was between 3 to 58 times more potent than gemcitabine in pancreatic cancer cell lines but doxorubicin was superior against both breast cancer cell lines compared to FUS.
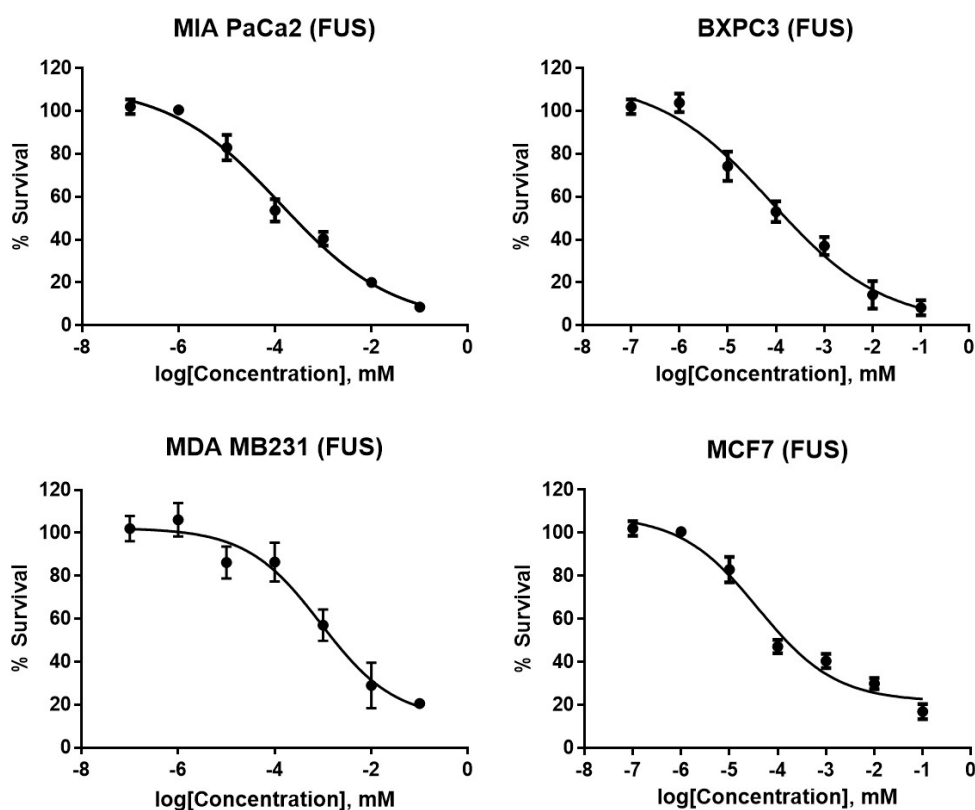
Figure 2. MTT cell-viability assay profile in pancreatic (MIA PaCa2 and BXPC3) and breast (MDA MB 231 and MCF7) cancer cell lines treated with FUS for 24 h.

The relatively rapid cytotoxicity observed for FUS during the cell culture experiments led us to carry out a time-dependent cytotoxicity assay by monitoring percentage survival after 4 and 8 hours post-incubation. FUS showed greater toxicity at both 4 and 8 hours at 4 x IC_{50} concentration in MIA PaCa2 cell line compared to gemcitabine. The differences were statistically significant (P<0.01) (Figure 3a). Similarly rapid toxicity was observed against MDA MB 231 cell line at 4h (P<0.03) and at 8h (P<0.01) compared to doxorubicin (ESI) although doxorubicin was notably more potent than FUS after 24h incubation.
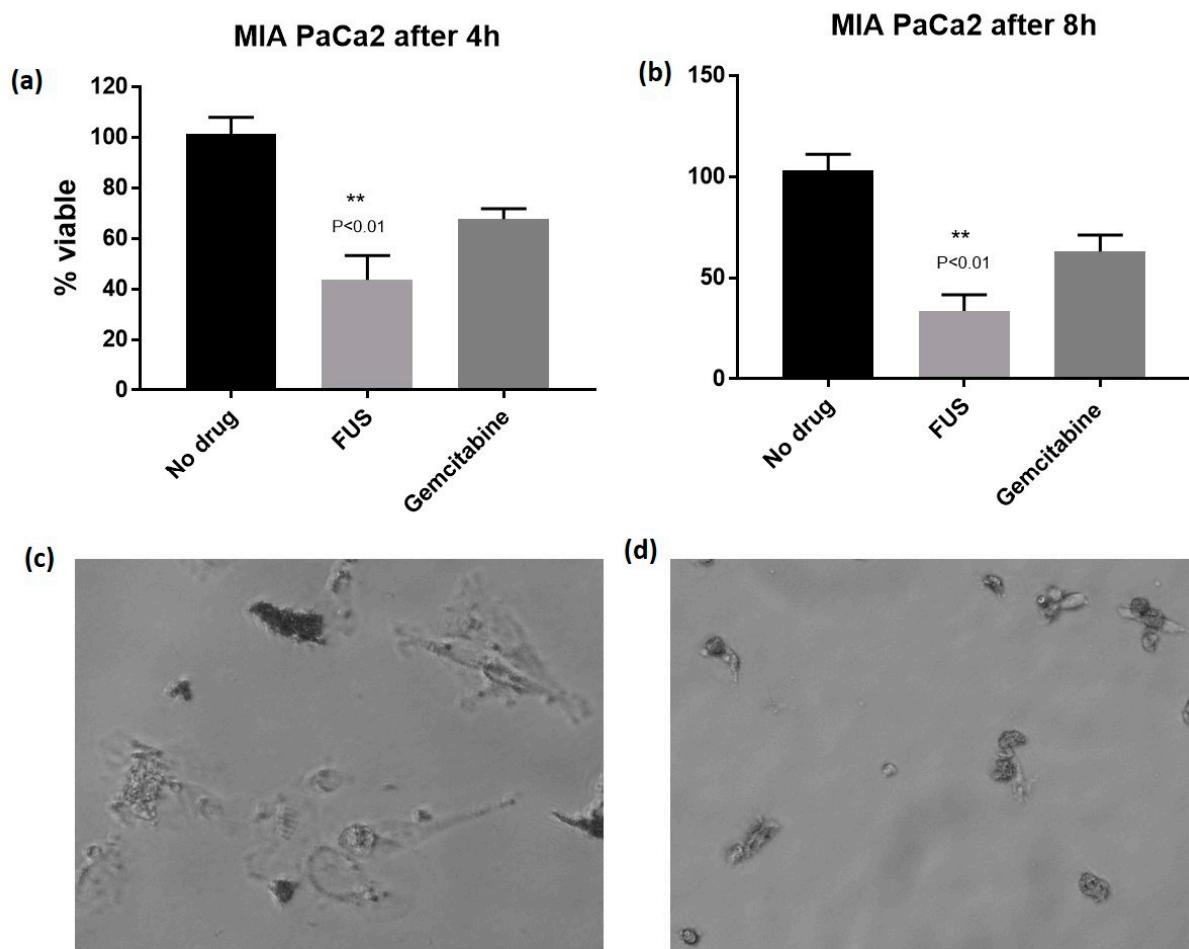
Figure 3.(a) & (b) FUS showed statistically significant rapid toxicity (P<0.01) against MIA PaCa2 cell line after 4h and 8h incubation; (c) & (d) morphological changes observed in MIA PaCa2 cell lines after 4h and 8h incubation, respectively.

The morphological changes in the MIAPaCa2 cell line after incubating with FUS was monitored using a Nikon TS100 inverted microscope fitted with a camera. The cells appeared to show both apoptotic and necrotic damages within 4 hours post-incubation and the damages were fully evident at the 8 hour time point (Figure 3c & d). These images along with the survival analysis points towards the ability of the compound to induce severe stress resulting in rapid toxicity against the cell lines. This rapid cytotoxicity is intriguing and potentially a useful characteristic for an anticancer scaffold that can be developed against pancreatic cancer. Further studies are required to ascertain the mechanism of action of this compound and will be reported in due course.

In summary, FUS is a known sesterterpene mycotoxin isolated from the endophytic fungus *F. solani*. This compound showed potent and rapid cytotoxicity against both pancreatic and breast cancer cell lines tested in this study. The complex structure and intriguing biological activity of FUS make it a good target for chemical synthesis and a lead structure for a medicinal chemistry project to develop a new anticancer drug against pancreatic cancer.

3. Experimental Section

3.1. Collection and Identification of the Plant Material

The aerial part of *A. hookerianum* was collected from Pablakhali, Rangamati, Chittagong Hill tracts, Bangladesh on August 10, 2014 and identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen of the plant has been deposited (Accession no.: DACB 40633) in the herbarium for further reference.

3.2. General Experimental Procedures

The NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using $CDCl_3$. The HRMS spectrum was recorded on an Exactive Orbitrap by a Thermo Scientific mass spectrometer at King's College London, (London, UK), and the data were processed by Thermo XCalibur 2.2. Column chromatography was carried out on silica gel (70−230 mesh and 230−400 mesh, Merck, Germany). Organic solvents, potato dextrose agar (PDA) medium, and TLC plates were purchased from Merck, Germany.

3.3. Isolation of Fungal Material

About 300 g of fresh and healthy parts of the plant (leaves, root, and petiole) was cut with a sterile scalpel and stored at 4°C in a sterile polyethene bag prior to use. Endophytic fungi were isolated from the fresh plant parts following the procedure, established at Pharmaceutical Sciences Research Division, BCSIR Laboratories, Dhaka, Bangladesh [11-14], Total four endophytic fungi were isolated from different parts of *A. hookerianum* bearing the internal strain no. AHPE-3, AHPE-4, AHLE-1 and AHLE-4. All the endophytic fungi were taxonomically identified up to genus level on the basis of macroscopic and microscopic morphological characters as *Fusarium*

sp. (AHPE-3), *Fusarium* sp. (AHPE-4), *Colletotrichum* sp. (AHLE-1) and *Colletotrichum*sp. (AHLE-4). The fungus AHPE-4 was selected for further investigation, based on the brine shrimp lethality bioassay data (SI), and was cultured at a large scale to isolate bioactive secondary metabolites.

3.4. Molecular Identification of the Endophytic Fungus AHPE-4

For identification and differentiation, the Internal Transcript Spacer regions (ITS4 and ITS5) and the intervening 5.8S rRNA region was amplified and sequenced using electrophoretic sequencing on an ABI 3730 x l DNA analyzer (Applied Biosystems, USA) using Big Dye Terminator v 3.1 cycle sequencing kit. The ITS regions of the fungus were amplified using PCR (Hot Start Green Master Mix, Promega, USA) and the universal ITS primers, ITS4 (5′- TCC GTA GGT GAA CCT GCG G-3′) and ITS5 (5′- GGA AGT AAA AGT CGT AAC AAG G -3′). The PCR products were purified and desalted using the Hot Start Green Master Mix (Cat: M7432, Promega, USA.) and sequenced on an ABI 3730 x l DNA analyzer (Applied Biosystems, USA). The sequences were aligned and prepared with the software Chromas (V 2.6.2) and matched against the nucleotide-nucleotide database (BLASTn) of the U.S. National Center for Biotechnology Information (NCBI) for final identification of the endophytic isolate. Finally, the sequence data (SI) were deposited in the Gen Bank database (accession number MG75792) which revealed 99% similarity other related fungal isolates of *F. solani* bearing accession numbers KX 497027, KJ863503, AB 190389, AY433805 *etc* deposited in NCBI.

3.5. Extraction of the Fungal Material and Isolation of FUS

The fungus *F. solani* (AHPE-4), isolated from the petiole of the plant *A. hookerianum* was cultivated at 28 ± 2°C for 28 days on potato dextrose agar (PDA). The culture media were extracted with ethyl acetate for seven days in an air-tight flat bottom container with occasional shaking and stirring. This procedure was repeated for three times to obtain the crude extract. The crude extracts of endophytic fungi was then filtered using sterilized cotton filter followed by Whatman no. 1 filter papers. The solvent was evaporated with a rotary evaporator at low temperature (40°C- 50°C) and reduced pressure.

The crude fungal extract (8 gm) was subjected to column chromatography for fractionation on silica gel (70-230 mesh) by using gradients of petroleum ether/ ethyl acetate, then ethyl acetate, followed by a gradient of ethyl acetate/ methanol, and finally methanol to afford a total of 15

fractions. These fractions were screened by TLC on silica gel under UV light and by spraying with vanillin-H_2SO_4 spray reagents. The column fraction of petroleum ether/ 15% ethyl acetate was subjected to preparative TLC on silica gel (toluene/ 20% ethyl acetate, 3 developments) to obtain FUS.

3.5.1 Fusaproliferin

18 mg, white, amorphous sticky mass; (^{1}H NMR, $CDCl_3$): δ 2.40 (1H, dd, J = 10.8, 13.6 Hz, H-1′), 1.74 (1H, m, H-1″), 5.27 (1H, dd, J = 5.0, 10.2 Hz, H-2), 2.30 (1H, m, H-4′), 2.06 (1H, m, H-4″), 2.30 (1H, m, H-5′), 2.11 (1H, m, H-5″), 5.15 (1H, bs, H-6), 2.11 (1H, m, H-8′), 1.82 (1H, d, J = 9.2 Hz, H-8″), 1.82 (1H, d, J = 9.2 Hz, H-9′), 1.65 (1H, m, H-9″), 4.08 (1H, dd, J = 3.4, 9.8 Hz, H-10), 5.40 (1H, bt, H-12), 2.40 (1H, dd, J = 10.8, 13.6 Hz, H-13′), 1.95 (1H, m, H-13″), 2.69 (1H, dd, J = 2.0, 11.2 Hz, H-14), 2.80 (1H, sextet, H-19), 1.66 (3H, s, H-20), 1.66 (3H, s, H-21), 1.59 (3H, s, H-22), 1.02 (3H, s, H-23), 4.31 (1H, dd, J = 8.0, 10.4 Hz, H-24′), 4.27 (1H, dd, J = 7.2, 10.4 Hz, H-24″), 1.33 (3H, d, J = 6.8 Hz, H-25), 2.06 (3H, s, H-27), 5.56 (1H, s, 17- OH). ^{13}C NMR: δ_C 39.1 (C-1), 121.4 (C-2), 138.2 (C-3), 40.3 (C-4), 23.8 (C-5), 124.3 (C-6), 132.9 (C-7), 34.9 (C-8), 29.7 (C-9), 76.5 (C-10), 136.5 (C-11), 128.9 (C-12), 28.7 (C-13), 49.6 (C-14), 49.0 (C-15), 207.9 (C-16), 147.3 (C-17), 146.7 (C-18), 33.7 (C-19), 15.5 (C-20), 15.3 (C-21), 10.4 (C-22), 16.2 (C-23), 66.4 (C-24), 14.5 (C-25), 170.9 (C-26), 20.9 (C-27). HRESIMS *m/z* 467.2778 [M +Na]$^+$ (calcd mass 467.2773, [$C_{27}H_{40}O_5$+ Na]$^+$).

3.6. Bioassays

3.6.1. Cell Culture

The MIA PaCa2 (pancreatic adenocarcinoma), BXPC3 (pancreatic adenocarcinoma), MDA-MB-231 (triple-negative breast cancer), MCF-7 (estrogen receptor positive breast cancer) cell lines were obtained from the American Type Culture Collection. TheMIA PaCa2 cell line was maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with fetal bovine serum (10% v/v; Invitrogen), horse serum (2.5% v/v; Invitrogen) and penicillin-streptomycin (1% v/v, Invitrogen). The BXPC3 cell line was maintained in RPMI-1640 medium (DMEM; Invitrogen), supplemented with fetal bovine serum (10% v/v; Invitrogen), and penicillin-streptomycin (1% v/v, Invitrogen). The MDA MB 231 cell line was maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with fetal bovine serum (10% v/v;

Invitrogen), l-glutamine (2 mM; Invitrogen), non-essential amino acids (1x; Invitrogen) and penicillin-streptomycin (1% v/v, Invitrogen). The MCF7 cell line was maintained in Eagle's Minimum Essential medium supplemented with fetal bovine serum (10% v/v; Invitrogen), 0.01 mg/ml human recombinant insulin and penicillin-streptomycin (1% v/v, Invitrogen). During seeding, cells were counted using a Neubauer hemocytometer (Assistant, Hanover, Germany) by microscopy (Nikon, Melville, NY, USA) on a non-adherent suspension of cells that were washed in PBS, trypsinized, centrifuged at 8°C at 8000 rpm for 5 min, and re-suspended in fresh medium.

3.6.2. MTT Assay

The cells were grown in normal cell culture conditions at 37ºC under a 5% CO_2 humidified atmosphere using an appropriate medium. The cell count was adjusted to 10^5 cells/mL and 2,500 cells (MDA-MB-231) or 5,000 cells (A4 and WI-38) were added per well. The cells were incubated for 24 h and 1 μL of the appropriate inhibitor concentrations was added to the wells in triplicate. After 96 h of continuous exposure to each compound, the cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Lancaster Synthesis Ltd, Morecambe, Lancashire, UK) colorimetric assay. Absorbance was quantified by spectrophotometry at λ = 570 nm (Envision Plate Reader, PerkinElmer, Waltham, MA, USA). IC_{50} values were calculated by a dose-response analysis using the Prism GraphPad Prism® software.

Supplementary Materials: The Supporting Information is available free of charge on the website, experimental procedures and ^{1}H, ^{13}C NMR, DEPT-135 and HRMS spectra of FUS (PDF).

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Conflict of Interest: The authors declare no competing financial interest.

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References

1. Kleeff, J.; Korc, M.; Apte, M.; La Vecchia, C.; Johnson, C. D.; Biankin, A. V.; Neale, R. E.; Tempero, M.; Tuveson, D. A.;Hruban, R. H. Neoptolemos, J. P. Pancreatic cancer. *Nat. Rev. Dis. Prim.* **2016**, *2*, 16022, doi: 10.1038/nrdp.2016.22.

2. Van Cutsem, E.; Vervenne, W. L.; Bennouna, J.; Humblet, Y.; Gill, S.; Van Laethem, J.-L.; Verslype, C., Scheithauer, W.; Shang, A.; Cosaert, J. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J. Clin. Oncol.* **2009**, *27*, 2231-2237, doi: 10.1200/JCO.2008.20.0238.

3. Siriwardena, A. K.; Siriwardena, A. M. Pancreatic cancer. *BMJ :Brit. Med. J.* **2014**, 349, doi: 10.1136/bmj.g6385.

4. Wong, M. C. S.; Jiang, J. Y.; Liang, M., Fang, Y.; Yeung, M. S.; Sung, J. J. Y. Global temporal patterns of pancreatic cancer and association with socioeconomic development. *Sci. Rep.* **2017**, *7,* 3165, doi: 10.1038/s41598-017-02997-2.

5. Vincent, A.; Herman, J.; Schulick, R.; Hruban, R. H.; Goggins, M. Pancreatic cancer. *The Lancet* **2011**, *378*, 607-620, doi: 10.1016/S0140-6736(10)62307-0.

6. Ouyang, L.; Luo, Y.; Tian, M.; Zhang, S. Y., Lu, R.;Wang, J. H.; Kasimu, R., Li, X. Plant natural products: from traditional compounds to new emerging drugs in cancer therapy. *Cell Prolif.* **2014**, *47*, 506-515, doi: 10.1111/cpr.12143.

7. Harvey, A. L.; Edrada-Ebel, R.; Quinn, R. J. The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Disc*. **2015**, *14*, 111-129, doi: 10.1038/nrd4510.

8. Kumar, A., Patil, D.; Rajamohanan, P. R.; Ahmad, A. 2013. Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. *PLoS ONE*. **2013**, *8*, e71805, https://doi.org/10.1371/journal.pone.0071805.

9. Nascimento, A. M. d.; Conti, R.; Turatti, I. C.; Cavalcanti, B. C.; Costa-Lotufo, L. V.; Pessoa, C.; Moraes, M. O. d.; Manfrim, V.; Toledo, J. S.; Cruz, A. K. Bioactive extracts and chemical constituents of two endophytic strains of *Fusarium oxysporum*. *Rev. bras. farmacogn.* **2012**, *22*, 1276-1281, doi: 10.1590/0074-02760140243.

10. Cui, Y.; Yi, D.; Bai, X.; Sun, B.; Zhao, Y.; Zhang, Y. Ginkgolide B produced endophytic fungus (*Fusarium oxysporum*) isolated from *Ginkgo biloba. Fitoterapia* **2012,** *83*, 913-920, doi: 10.1016/j.fitote.2012.04.009.

11. Khan, M.I.H.; Sohrab, M.H.; Rony. S. R.; Tareq, F.S.; Hasan, C.M.; Mazid, M.A.; Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. *Toxicol. Rep.* **2016**, *3*, 861–865, doi: 10.1016/j.toxrep.2016.10.005.

12. Chowdhury, N.S.; Sohrab, M.H.; Rony. S. R.; Sharmin, S.; Begum, M,N.; Rana, M.S.; Hasan, C. M. Identification and bioactive potential of endophytic fungi from *Monochoria hastata* (l.) Solms. *Bangladesh J. Bot.* **2016**, *45*, 187-193.

13. Chowdhury, N. S.; Sohrab, M. H.; Rana, M. S.; Hasan, C. M.; Jamshidi, S.; Rahman, K. M., Cytotoxic naphthoquinone and azaanthraquinone derivatives from an endophytic *Fusarium solani*. *J. Nat. Prod.* **2017**, *80*, 1173-1177, doi: 10.1021/acs.jnatprod.6b00610.

14. Khan, N.; Afroz, F.; Begum, M,N.; Rony. S. R.; Sharmin, S.; Moni, F.; Hasan, C. M.; Shaha, K.; Sohrab, M.H. Endophytic *Fusarium solani*: A rich source of cytotoxic and antimicrobial napthoquinone and aza-anthraquinone derivatives, Toxicol. Rep. **2018**, *5*, 970-976,   DOI: 10.1016/j.toxrep.2018.08.016

15. Takemoto, K.; Kamisuki, S.; Chia, P. T.; Kuriyama, I.; Mizushina, Y.; Sugawara, F. Bioactive dihydronaphthoquinone derivatives from *Fusarium solani*. *J. Nat. Prod.* **2014**, *77*, 1992-1996, doi: 10.1021/np500175j.

16. Shweta, S.; Zuehlke, S.; Ramesha, B. T.; Priti, V.; Kumar,M.P.; Ravikanth, G.; Spiteller, M.; Vasudeva, R.; Shaanker, U. R. Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. *Phytochemistry* **2010**, *71*, 117-122, doi: 10.1016/j.phytochem.2009.09.030.

17. Chakravarthi, B.; Das, P.; Surendranath, K.; Karande, A. A.; Jayabaskaran, C. Production of paclitaxel by *Fusarium solani* isolated from *Taxus celebica*. *J. Biosci.* **2008**, *33*, 259-267.

18. Randazzo, G.; Foglianoa, V.; Ritienia, A.; Rossin, L.M.E.; Scarallo A.; Segred, A.L. Proliferin, a new sesterterpene from *Fusarium proliferatum. Tetrahedron* **1993**, *49*, 10883-10896.

19. Fotso, J.; Leslie, J.F.; Smith, S, J. Production of beauvericin, moniliformin, fusaproliferin, and fumonisins B1, B2, and B3 by fifteen ex-type strains of *Fusarium* Species, *Appl. Environ. Microbiol*. **2002**, *68*, 5195–5197, doi: 10.1128/AEM.68.10.

20. Ritieni, A.; Monti, S.M.; Moretti, A.; Logrieco, A.; Gallo, A.; Ferracane, R.; Fogliano, V. Stability of fusaproliferin, a mycotoxin from *Fusarium* spp. J. Sci. Food Agric. **1999**, *79*, 1676–1680.

21. Logrieco, A.; Moretti, A.; Fornelli, F.; Fogliano, V.; Ritieni, A.; Caiaffa, M.F.; Randazzo, G.; Bottalico, A.; Macchia, L. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina,* SF-9 insect cells, and IARC/LCL 171 human B lymphocytes. *Appl. Environ. Microbiol*. **1996**, *62*, 3378–3384.

22. Ritieni, A.; Monti, S.M.; Randazzo, G.; Logrieco, A.; Moretti, A.; Peluso, G.; Ferracane, R.; Fogliano, V. Teratogenic effects of fusaproliferin on chicken embryos. *J. Agric. Food Chem*. **1997a**, *45*, 3039–3043.