Journal of Biologically Active Products from Nature

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbap20

Antibacterial and Cytotoxic Compounds from Endophyte *Fusarium solani* isolated from *Centella asiatica* (L.)

Fatema Moni, Naki Saifullah, Farhana Afroz, Satyajit Roy Rony, Suriya Sharmin, ADA Shahinuzzaman, Muhammad Abdullah Al-Mansur, Sharif Md. Al-Reza & Md. Hossain Sohrab

To cite this article: Fatema Moni, Naki Saifullah, Farhana Afroz, Satyajit Roy Rony, Suriya Sharmin, ADA Shahinuzzaman, Muhammad Abdullah Al-Mansur, Sharif Md. Al-Reza & Md. Hossain Sohrab (2022): Antibacterial and Cytotoxic Compounds from Endophyte *Fusarium solani* isolated from *Centella asiatica* (L.), Journal of Biologically Active Products from Nature, DOI: 10.1080/22311866.2022.2144947

To link to this article: https://doi.org/10.1080/22311866.2022.2144947

Published online: 29 Nov 2022.

Taylor & Francis
Taylor & Francis Group
ISSN: (Print) 2231-1866; ISSN: (Online) 2231-1874

Journal of Biologically Active Products from Nature
https://www.tandfonline.com/loi/tbap

Original Article

Antibacterial and Cytotoxic Compounds from Endophyte *Fusarium solani* isolated from *Centella asiatica* (L.)

Fatema Moni [1], Naki Saifullah [2], Farhana Afroz [1], Satyajit Roy Rony [1], Suriya Sharmin [1], ADA Shahinuzzaman [1], Muhammad Abdullah Al-Mansur [3], Sharif Md. Al-Reza [2], Md. Hossain Sohrab [1]*

[1] Pharmaceutical Sciences Research Division, BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh
[2] Department of Applied Chemistry and Chemical Engineering, Islamic University, Kushtia - Jhenaidah, Bangladesh
[3] Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh
* Corresponding Author: mhsohrab@bcsir.gov.bd (Md. Hossain Sohrab)

Received 02 August 2022; Received in revised form 01 November 2022; Accepted 03 November 2022

Abstract: The antimicrobial and cytotoxic activity of metabolites isolated from an endophyte *Fusarium solani* were evaluated which was associated with the medicinal plant *Centella asiatica* Linn.. Repeated chromatographic separations of ethyl acetate extract of *Fusarium solani* lead to the isolation of three naphthoquinone Anhydrofusarubin **(1)**, Fusarubin **(2)**, Javanicin **(3)** and one aza-anthraquinone 5-deoxybostrycoidin **(4)**. The structures of isolated compounds were determined by analysis of NMR spectrum data and comparison of spectroscopic data with those of known compounds. The cytotoxic activity of isolated compounds was tested against the Human Lung cancer cell line and antimicrobial activity by disc diffusion method. Significant antibacterial activity and Cytotoxicity of the isolated secondary metabolites suggested that Azaanthraquinones and Napthaquinones are potential lead to develop antibacterial and anticancer drugs.

Keywords: *Centella asiatica* Linn, *Fusarium solani*, Naphthoquinone, Aza-anthraquinone, Antibacterial activity, Cytotoxic activity.

Introduction:

Now a day's cancer is a common and frequently-occurring disease which threatens human health seriously. When some of the body's cells grow uncontrollably and spread into other parts of the body with continuous self-renewal and reproduction, it is defined as cancer [1].

Several treatment plans are followed now a day's for cancer treatment like surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapy [2]. Considering cancer as a systemic disease, chemotherapy and targeted therapy are used to kill cancer cells that have metastasized [3]. Since the discovery of chemotherapy, scientists have developed various drugs for the successful treatment of this ailment but the effective treatment of the cancer is hampered by the development of resistance and side effects.

The discovery of antibiotics is a revolution as their clinical availability has led to control of bacterial disease and a decrease of mortality and mortal dramatically. Antibiotics are also used for the treatment of cancer. It is evident from

research findings that antibiotics can promote apoptosis, inhibit cancer growth and prevent metastasis. For these reasons, antibiotics are also increasingly being used to assist in cancer treatment [2]. Besides these, there is an increased risk of infection due to low white blood cell count (neutropenia) while patients are receiving chemotherapy which is caused by a toxic effect of chemotherapy on bone marrow. Standard antibiotic regimens for cancer patients with neutropenia and fever are directed at most of the bacteria that can cause infections [4]. It is also reported that Antibiotic prophylaxis in afebrile neutropenic patients significantly reduced all-cause mortality [5]. Despite the significant role of antibiotics in modern lifestyle, their excessive use has created strong selective pressure on microorganisms leading to develop antibiotic resistance mechanisms for their preferential survival. The current drug discovery output is not delivering new antibacterial entities at a rate that is sufficient to battle against present situations of antibiotic resistance [6].

Endophytic fungi are microorganisms either bacteria or fungi, that colonize inside the healthy tissues of the host plants without causing any apparent symptoms of diseases. Endophytic fungi are a valuable source for the production of valuable bioactive compounds such as anticancer, antioxidant, antimicrobial, antidiabetic and industrial enzymes, etc. [7-12]. A recent study by Zhou *et. al*. reported prominent antimicrobial activity of fungal extract against gram-positive bacteria *Enterococcus faecalis* (EF), gram-negative bacteria *Pseudomonas adaceae* (PA), methicillin-resistant *Staphylococcus aureus* (MRSA) and pathogenic fungus *Monilia albicans* (MA) after the screening of forty-six fungal endophytes isolated from mangrove plant. The study also reported cytotoxic activity of the fungal extracts against A549 human lung cancer cells, HepG2 human hepatocellular cells, HeLa human cervical carcinoma cells, and HepG2 human hepatocellular cells [13]. Moreover, endophyte *Fusarium solani* was reported to produce antimicrobial metabolites [13,14] comment 1) and cytotoxic metabolites against cancer cell lines like Vero cell line, human alveolar adenocarcinoma cell (A549) [14,15].

Certain endophytic fungi are capable to synthesize the medicinal products produced similarly in plants. For this reason, researchers are focusing on the isolation of endophytic fungi from medicinal plants and uncapping the vast number of endophytic fungi species, some of which have the potential to be used in the production of medicine [16]. Thus present study aimed to isolate secondary metabolites from endophytic fungi, *Fusarium solani*, isolated from the medicinal plant *Centella asiatica* (Linn) and investigate their cytotoxic and antimicrobial activity.

Experimental

General experimental procedures

The Nuclear Magnetic Resonance (NMR) spectra were recorded by 400 MHz spectrometers (Bruker, Switzerland). The sample was prepared using deuterated dichloromethane to take NMR spectra. The δ values for ^{1}H and ^{13}C spectra were referenced relative to the residual non-deuterated solvent signal. Column chromatography was carried out on silica gel (70-230 and 230-400 mesh, Merck, Germany). Thin layer chromatography (TLC) and preparative Thin Layer Chromatography (PTLC) were performed with aluminium-backed plates coated with silica gel F254. Organic solvents, potato dextrose agar (PDA) medium, and TLC plates were purchased from Merck, Germany.

Plant material

The fresh, healthy and mature plant was collected from the Bangladesh Council of Scientific and Industrial Research (BCSIR) campus, Dhanmondi, Dhaka in March, 2016. After collection, the plant material was identified from Bangladesh National Herbarium (BNH), Dhaka, Bangladesh. A voucher specimen of the collected plant sample was deposited and maintained at the herbarium under the accession number DACB – 42998 for further reference. A taxonomist at Bangladesh National Herbarium (BNH), Dhaka, Bangladesh, recognized and verified the plant material. Following the method previously established at Pharmaceutical Sciences Research Division, BCSIR Laboratories, Dhaka, Bangladesh,

Endophytic fungi were isolated from the fresh plant parts after surface sterilization [17].

Identification of fungal material

The fungal strain, *Fusarium solani* (Figure 1) was identified by DNA amplification following a molecular biology protocol where the internal transcriber spacer region was sequenced (ITS) [18].

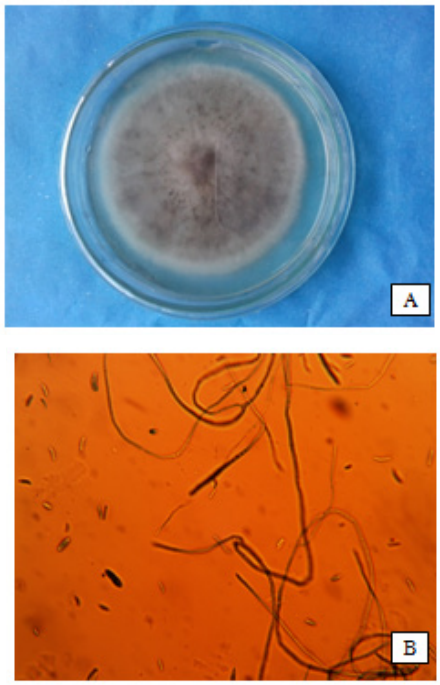

Figure 1. *Fusarium solani* isolated from *Centella asiatica* (L.) A. Macroscopic view on PDA culture media B. Microscopic view

For identification, the fungus was cultivated on Potato dextrose (PDA) agar medium. In an Eppendorf tube, fungus hyphae were collected and lyophilized. Glass beads were used to break apart and disrupt the lyophilized fungal mycelia. Following the manufacturer's instructions, fungal DNA was extracted using the DNeasy Plant Mini Kit (QIAgen, USA). As the internal transcribed spacer (ITS) region of nuclear DNA has been selected as a standard marker for fungal DNA barcoding, the Internal Transcribed Spacer regions (ITS4 and ITS5) and the intervening 5.8S rRNA region was chosen for amplification and sequencing. For amplification of isolated DNA, the Polymerase Chain Reaction (PCR) was carried out using HotStarTaq Master Mix Kit (QIAgen, USA) using ITS4 (50-TCC GTA GGT GAA CCT GCG G-30) and ITS5 (50-GGA AGTAAA AGT CGT AAC AAG G-30) (Invitrogen, USA) as primers. Isolated DNA templates and primers were mixed with HotStarTaq Master Mix Kit in a total volume of 50 µL and placed in a thermal cycler (BioRad, USA) for reaction and amplification of DNA. The PCR product was purified using the Gel Electrophoresis technique. 2% Agarose-Gel Electrophoresis stained with 1% ethidium bromide was used at 75 V for 60 minutes in 1 X TBE buffer. The stained DNA fragment with an approximate size of 550 bp was then excised from the agarose gel and further purified using Perfect Prep Gel Cleanup Kit (Eppendorf, USA) by following the manufacturer's protocol. The PCR-amplified and purified fungal DNA was then sent to a commercial service provider for sequencing and the base sequence was compared with publically accessible databases like GenBank using Blast-Algorithmus. The sequence was eventually deposited in the National Center for Biotechnology Information (NCBI) of the United States (accession number OL307933-OL307934) which revealed 95.75% similarity (Query cover 98%) to another fungal isolate with *Fusarium solani* (accession number NR_163531.1) deposited in the U.S National Center for Biotechnology Information (NCBI).

Chemical investigation of fungal material

Cultivation and extraction

The fungal strain *Fusarium solani* (internal strain no. CnABE-2) was isolated from the stem bark of *Centella asiatica*L.which was isolated following surface sterilization of the plant part. On potato dextrose agar medium, the fungal strain was grown for 28 days at a temperature of 2°C**.** Around 28 days, fungal hyphae were observed to die off and the back side colour of the culture plate was changed indicating the secretion of secondary metabolites by fungi. The culture media, potato dextrose agar, was extracted with ethyl acetate to produce the crude extract when the incubation period was complete (1.20g) [19].

Isolation of compounds

For isolation of pure compounds, the crude extract was subjected to column chromatography using silica gel as a stationary phase running with a gradient mobile phase started with n-hexane, followed by mixtures of n-hexane-Dichloromethane, then with mixtures of dichloromethane-methanol with increasing polarity and finally with methanol. A total of 65 fractions were collected while the collection volume was 50 mL of each. According to their TLC patterns, similar fractions were mixed which yielded 15 factions from which pure compounds were isolated. Different techniques like solvent treatment, PTLC were used for the purification of compounds from column fractions. Compound 1 (10.50 mg) was isolated from a combined column fraction (Column fraction 9) with the eluent system n-hexane / 5% CH2Cl2. From TLC behaviour it was found in almost pure form which was further purified by different solvent treatments. It was obtained as a violet crystal. Column fraction 44 eluted with Dichloromethane/ 1.2% methanol was also obtained with slight impurity. This fraction was further purified by repeated solvent treatment and designated as Compound 2 (8.50 mg). It was obtained as a red-orange-coloured crystal. The combined column fraction of 42-43 eluted with Dichloromethane/ 1% Methanol was subjected to Preparative Thin Layer Chromatography (PTLC) (stationary phase: silica gel F254, mobile phase: toluene-10% ethyl acetate, the thickness of plates: 0.5 mm) for further separation of compounds using a solvent system of toluene-10% ethyl acetate as mobile phase. From the developed PTLC plate few bands were observed. Among them, two sets of bands were scraped off from the generated PTLC plate independently, extracted with ethyl acetate, and then eluted by cotton filtering. One band (Rf=0.45 with 10% ethyl acetate in toluene) produced red amorphous powder when the solvent was evaporated, was designated as compound 3 (9.5 mg). The other band with Rf=0.42 value (10% ethyl acetate in toluene) produced violet-coloured crystal, which was designated as compound 4 (2.0 mg). The NMR data of isolated compounds were analyzed to elucidate the structures (1H NMR & 13C NMR) and compared with the previously published data of those compounds.

Compound 1, Anhydrofusarubin: Violet solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.02 (1H, s, 5-OH), 12.63 (1H, s, 10-OH), 6.15 (1H, s, H-8), 5.97 (1H, s, H-4), 5.20 (2H, s, H-1), 3.90 (3H, s, 7-OCH_3), 2.00 (3H, s, 3-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.92 (C, C-9), 177.84 (C, C-6), 161.58 (C, C-3), 160.0 (C, C-7), 157.86 (C, C-10) 157.82 (C, C-5), 133.06 (C, C-4a), 122.76 (C, C-10a), 110.95 (C, C-5a), 109.97 (C, C-8), 107.99 (C, C-9a), 94.72 (C, C-4), 62.97 (CH_2, C-1), 56.69 (CH_3, C-7-OCH_3), 20.13 (CH_3, C-3-CH_3); ^{1}H NMR and ^{13}C NMR data were consistent with reported data [20,21].

Compound 2, Fusarubin: Orange red solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.66 (1H, s, 5-OH), 12.93 (1H, s, 10-OH), 6.17 (1H, s, H-8), 4.88 (2H, s, H-1), 3.92 (3H, s, 7-OCH_3), 3.03 (1H, d, J = 18.2, Ha-4), 2.70 (1H, d, J = 18.2, Hb-4), 2.25 (1H, bs, 3-OH), 1.65 (3H, s, 3-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.84 (C, C-9), 178.34 (C, C-6), 160.73 (C, C-10), 160.73 (C, C-5), 157.23 (C, C-7), 132.89 (C, C-10a), 137.16 (C, C-4a), 109.70 (C, C-5a), 109.70 (C, C-8), 107.64 (C, C-9a), 94.30 (C, C-3), 32.20 (C, C-4), 58.58 (CH_2, C-1), 56.78 (CH_3, C-7-OCH_3), 29.40 (CH_3, C-3-CH_3); ^{1}H NMR and ^{13}C NMR data were consistent with reported data [20,21].

Compound 3, Javanicin: Red solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.25 (1H, s, 4-OH), 12.85 (1H, s, 9-OH), 6.20 (1H, s, H-7), 4.02 (3H, s, 6-OCH_3), 3.92 (2H, s, H-3), 2.29 (3H, s, H-11), 2.23 (3H, s, H-10). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.84 (C, C-2), 161.4 (C, C-6), 160.6 (C, C-9), 160.3 (C, C-4), 142.5 (C, C-3a), 134.2 (C, C-9a), 109.6 (CH, C-7), 108.4 (C, C-8a), 107.9 (C, C-4a), 56.7 (CH_3, C-6-OCH_3), 31.9 (CH_2, C-3), 29.4 (CH_3, C-11), 12.9 (CH_3, C-10); ^{1}H NMR and ^{13}C NMR data were consistent with reported data [22].

Compound 4, 5 –deoxybostrycoidin: Violet solid**;** ^{1}H NMR (400 MHz, $CDCl_3$): δ2.79 (3H, s, 2-C H 3); 4.01 (3H, s, 6-O C H 3); 6.75 (1H,

s, 7-H); 7.50 (1H, s 5-H); 7.95 (1H, s 3-H); 9.49 (1H, s, H-10); 13.20 (1H, s, 8-OH) [23].

Bioassay screening

Antimicrobial screening

The isolated compounds were screened for antimicrobial activity by the disc diffusion method described by Bauer *et al.* [24]. The antimicrobial activity was tested against two gram-negative pathogenic bacteria like *Escherichia coli* (ATCC 28,739) & *Salmonella typhi* (ATCC 28,633), two gram-positive bacteria like *Staphylococcus aureus* (ATCC 25,923) & *Bacillus megaterium* (ATCC 18,812) and one fungal strain *Aspergillus niger.*

A stock solution of pure compounds was prepared by dissolving 1.0 mg of compounds into 100 µL of dichloromethane. 10 µL of stock solution was then impregnated into blank sterile discs with 7 mm of diameter. The discs were dried properly before placing into inoculated media with bacteria or fungus. After inoculation, the culture was kept in a normal freezer (6ºC) for 24 hours to facilitate diffusion and finally incubated for 18-24 hours for bacteria at 37°C and 48-96 hours at 28°C for fungi. To measure the antimicrobial activity, the zones of growth inhibition around the discs were measured and the assay was repeated three times. Disc of solvent (dichloromethane) was used as negative control while standard disc with kanamycin (30 µg/disc) and ketoconazole (30 µg/disc) was used as a positive control. Results were expressed as the mean of zones of inhibition in millimetres (mm) with standard deviation.

Cytotoxicity test

Cytotoxic activity of isolated compounds was tested against a Human Lung Cancer cell line by observing anti-proliferative activity using Trypan Blue Exclusion Method [21,25]. Lungs Cancer cells were grown in Dulbecco's Modified Eagles Medium (DMEM) augmented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin/ streptomycin/neomycin (100 U/mL), (0.1 mg/ mL) and 25mM HEPES in 5% (v/v) CO2, pH 7.4 at 37°C [26]. A stock solution of pure compounds was prepared in DMSO with a concentration of (1.0 mg/mL). The prepared doses(1 µg/mL, 5 µg/mL, 15 µg/mL & 20 µg/mL) were then administered to 1-day-old cultured cells. The cultured flask was incubated for 24 hours after the administration of doses. 1.0 mL of cell suspension was collected for cell counting after harvesting with 0.5% Trypsin. The harvested cells were stained with Trypan blue and the number of viable cells/dead cells were counted by using an automated cell counter (LUNA-II™, analytikjena, SouthKorea) [27]. Results were expressed as a percentage of dead cells in the following way:

% Dead cells = No of stained (dead) cells / Total number of cells X 100

Statistical analysis

All values for antimicrobial evaluation were expressed as mean±standard deviation (SD) using MS-Excell where n=3. For the cytotoxicity test of pure compounds, continuous variables were compared between groups using one-way analysis of variance (ANOVA) with a post hoc Tukey's test for the determination of the statistical significance using GraphPad Prism software. The results were considered statistically significant when $p<0.001$.

Results and discussions

Chemical investigation of ethyl acetate extract of *Fusarium solani* leads to the isolation of three naphthoquinone Anhydrfusarubin (1) (10.50 mg), Fusarubin (2) (8.50 mg), Javanicin (9.50 mg) (3) and one azaanthraquinone 5-deoxybostrycoidin (4) (2.0 mg) (Figure 2). To determine the structure of the isolated compounds, NMR spectra were analyzed and compared their spectroscopic data with those published in the literature.

Compound 1: It was isolated from a combined column fraction (Fraction no. 8-17) with the eluent system n-hexane / 5% CH_2Cl_2. It was obtained as a violet crystal. It appeared as a purple spot on the TLC plate (R_f = 0.71, Toluene / 10% EtOAc) and showed a dark quenching spot under UV light at 254 nm. It is soluble in dichloromethane, chloroform sparingly soluble in methanol and insoluble in n-hexane and petroleum ether.

In the ^{1}H NMR spectrum, two one proton singlets at δ 13.02 and 12.63 ppm, two one proton

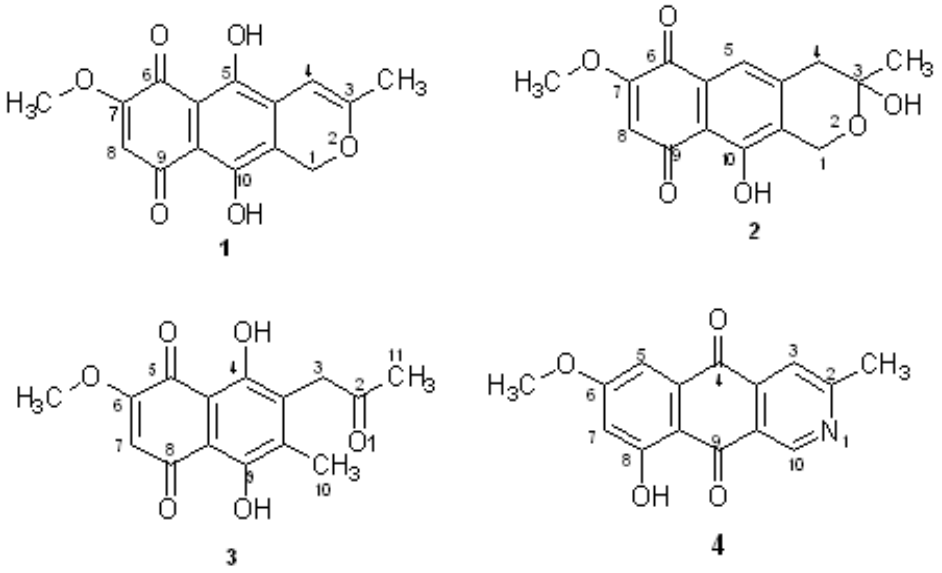

Figure 2. Structure of isolated compounds 1) Anhydrofusarubin 2) Fusarubin 3) Javanicin 4) 5-deoxybostrycoidin

singlet at 6.15 ppm & 5.97 ppm, two- proton singlets at 5.20 ppm, two three-proton singlets at 3.90 ppm and 2.00 ppm were found.

The singlet at δ = 2.0 ppm (integrating for three-proton) in the ^{1}H NMR (400 MHz, $CDCl_3$) and δ = 20.13 ppm in the ^{13}C NMR (100 MHz, $CDCl_3$) spectra could be attributed to one methyl group. The presence of three-proton singlet at δ = 3.90 ppm in the ^{1}H NMR and resonance at δ = 56.69 ppm in the ^{13}C NMR spectra could be characterized for the presence of methoxy group in compound **1**. Two olefinic protons could be identified from the resonances of two one-proton singlets at 5.97 and 6.15 ppm in the ^{1}H NMR and resonances at 94.72 and 109.97 ppm, respectively in the ^{13}C NMR spectra. The presence of two equivalent aliphatic protons was suggested by the presence of one two-proton singlet at δ = 5.20 ppm in the ^{1}H NMR and δ = 62.97 ppm in the ^{13}C NMR spectra.

Two sharp one-proton singlets were found at a relatively deshielded region with the chemical shift at δ=12.63 ppm and δ = 13.02 ppm in the ^{1}H NMR spectrum indicating the presence of two phenolic chelated hydroxyl groups which were supposed to form intramolecular hydrogen bond. Furthermore, the chemical shifts of the adjacent carbons of these two protons shifted downfield and were found at 157.82 ppm and 157.86 ppm which were also indicative of the presence of the phenolic hydroxyl group. Besides these, the evidence of a quinone system fused to the aromatic ring is suggested by the resonance at a relatively deshielded region at δ = 177.84 ppm and δ = 182.92 ppm in the ^{13}C NMR spectrum. Finally, by comparing the NMR data with those published in the literature, compound **1** was confirmed as Anhydrofusarubin [20,21].

Compound 2: It was isolated from a column fraction with the eluent system CH_2Cl_2/ 1.2% methanol. It was obtained as an orange-red-coloured crystal. It appeared as an orange spot on the TLC plate (R_f = 0.25, Toluene / 10% EtOAc). It showed a dark quenching spot under UV light at 254 nm. It is soluble in dichloromethane, and chloroform, sparingly soluble in methanol and insoluble in n-hexane, and petroleum ether.

In the ^{1}H NMR spectrum, two one proton singlets at *δ* 12.93 and 12.66 ppm, one proton singlet at 6.17 ppm, two proton singlet at 4.88 ppm, one three proton singlet at 3.92, two one proton doublet at 3.03 ppm & 2.70 ppm, one proton singlet at 2.25 ppm and three proton singlet at 1.65 ppm.

The presence of one methyl group is suggestive by the three-proton singlet at δ= 1.65 ppm in the ^{1}H NMR (400 MHz, $CDCl_3$) and resonance at δ= 29.4 ppm in the ^{13}C NMR spectra. In the ^{1}H

NMR spectrum, two one-proton doublets were found — one at δ= 2.70 ppm and the other at δ= 3.03 ppm. These two aliphatic protons were identified as geminal protons from their high coupling constant (J = 18.2 Hz) and resonance at δ= 32.2 ppm in the 13C NMR spectrum. One methoxy group is evident from the three-proton singlet at δ= 3.92 ppm in the ^{1}H NMR and peak at = 56.78 ppm in the ^{13}C NMR spectra. One two-proton singlet that was found at 4.88 ppm in the ^{1}H NMR and 58.58 ppm in the ^{13}C NMR spectra may be attributed to having one methylene group which is relatively deshielded may be due to an adjacent electronegative moiety. The presence of one - olefinic proton is suggestive due to the presence of resonance at δ = 6.17 ppm (one-proton singlet) in the ^{1}H NMR and δ = 109.7 ppm in the ^{13}C NMR spectra.

Two sharp one-proton singlets were found at a relatively deshielded region with the chemical shift at δ=12.66 ppm and δ = 12.93 ppm in the ^{1}H NMR spectrum indicating the presence of two phenolic chelated hydroxyl groups which were supposed to form intramolecular hydrogen bond. Furthermore, the chemical shifts of the adjacent carbons of these two protons shifted downfield and were found at 160.73 ppm and 160.73 ppm which were also indicative of the presence of the phenolic hydroxyl group. Besides these, the evidence of a quinone system fused to the aromatic ring is suggested by the resonance at a relatively deshielded region at δ = 178.34 ppm and δ = 184.84 ppm in the ^{13}C NMR spectrum. Finally, by comparing the NMR data with those published in the literature, compound **2** was confirmed as Fusarubin [20,21].

Compound 3: It was isolated from the column fraction by elution with Dichloromethane/MeOH. It was obtained as a red amorphous powder. It appeared as a red spot on the TLC plate and a dark quenching spot (R_f=0.45, Toluene/10% Ethyl acetate) under UV light at 254 nm and also exhibit blue fluorescence at 365 nm. *Compound* 3 is soluble in dichloromethane.

In the ^{1}H NMR spectrum, two one proton singlets at *δ* 13.25 and 12.85 ppm, one proton singlet at 6.20 ppm, three proton singlets at 4.02 ppm, one two proton singlet at 3.92, two three-proton singlet at 2.29 ppm & 2.23 ppm were found.

Resonances at 2.29 ppm (3H singlet) and 2.23 ppm (3H singlet) in the ^{1}H NMR spectrum and 29.4 ppm and 12.9 ppm in the ^{13}C NMR spectrum may be suggestive of the presence of two methyl groups. One methoxy group was supposed to be present as there is a peak at *δ* 4.02 ppm (3H singlet) in the ^{1}H NMR and *δ* 56.7 ppm in the ^{13}C NMR spectra. One methylene group is evident from the resonance of one two - proton singlet at 3.92 ppm in the ^{1}H NMR spectrum and 31.9 ppm in the ^{13}C NMR spectrum. One olefinic proton may be present in compound **3** as there is a one-proton singlet at 6.20 ppm in the ^{1}H NMR and resonance at 109.60 ppm in the ^{13}C NMR spectra. Two sharp proton singlets were found at a relatively deshielded region with the chemical shift at δ = 12.85 ppm and δ = 13.25 ppm in the ^{1}H NMR spectrum indicating the presence of two phenolic chelated hydroxyl groups which were supposed to form intramolecular hydrogen bond. Finally, by comparing the NMR data with those published in the literature, compound **3** was confirmed as Javanicin [22].

Compound 4: It was isolated from the column fraction by elution with Dichloromethane/MeOH. It was obtained as a violet amorphous powder. It appeared as a dark quenching spot on the TLC plate (R_f=0.42, Toluene/10% Ethyl acetate) under UV light at 254 nm. Compound 4 is soluble in dichloromethane.

Five one-proton singlets at 13.20, 9.49, 7.95, 7.50 and 6.75 ppm, two 3H singlets at 2.79 and 4.01 ppm were found in the 1H NMR spectrum. Resonances at 2.79 ppm (3H singlet) in the 1H NMR spectrum may be suggestive of the presence of one methyl group. One methoxy group was supposed to be present due the the presence of δ = 4.01 ppm (3H singlet) in the 1H NMR spectrum. The resonances at 9.49, 7.95, 7.50 and 6.75 ppm may be suggestive of the presence of four aromatic protons. The proton resonance at δ = 9.49 ppm was supposed to present in a nitrogen-containing aromatic ring as it shows resonance at more deshielded region. The predicted location of this proton's attachment was C-10, which is directly connected to the

nitrogen atom. The one sharp proton singlet was found at a relatively deshielded region with the chemical shift at δ = 13.20 ppm in the ^{1}H NMR spectrum indicating the presence of one phenolic chelated hydroxyl group which was supposed to form intramolecular hydrogen bond. By comparing the NMR data with those published in the literature, compound **4** was confirmed as 5-deoxybostrycoidin [23].

Antimicrobial activity test

The antimicrobial activity of isolated compounds was summarized in Table 1 (Figure 3). The zone of inhibition produced by the isolated compounds Anhydrofusarubin (Compound 1), Fusarubin (Compound 2), Janvanicin (Compound 3) and 5 -deoxybostrycoidin (Compound 4) was found to be 9.3-16.1 mm, 13.1-25.2 mm and 8.3-11 mm and 7.2-18.4mm respectively.

Among the isolated compounds *Fusarubin* exhibited highly significant antibacterial activity against four tested pathogenic bacteria *Bacillus megatorium, Staphycoccus aureus, Salmonella typhi* and *Escherichia. coli*. Anhydrofusarubin, Javanicin and 5-deoxybostrycoidin exhibited prominent antibacterial activity against the above-tested pathogenic bacteria. On the other hand, all of the compounds showed resistance against the tested fungi *Aspergillus niger* at the supplied doses. The study of antibacterial activity of isolated compounds will help to develop antibacterial drug in future.

Fusarium solani belonging to the fungal genus *Fusarium* is one of the most important endophytes having prominent biological and chemical diversity. Most of the compounds isolated from *Fusarium solani* belong to the chemical class Quinones, Napthaquinones and Azaanthraquinone with several bioactivities like antifungal, antiparasitic, antibacterial and anticancer activity [20,21,28,29,30].

Naphthoquinones and aza-anthraquinones are reported to exert both antibacterial and antifungal activity, but all of them are not active against all types of organisms, it depends on the structure of specific compounds. The antimicrobial properties of these four compounds **(1-4)** are also in agreement with the earlier reports on isolated naphthoquinones isolated from *F. solani* [31,32]. Though Ammar et. al., 1979 reported the mild antifungal activity of Anhydrofusarubin against *A. niger* [3], our study is consistent with our previous report [21].

Naphthoquinone and aza-anthraquinones are reported to exert their antibacterial activity with a definite mode of action **34**. It is reported that many Naphthoquines and its derivatives exhibit their antibacterial activity by bacterial membrane binding, generation of reactive oxygen species in bacteria, plasmid curing/inactivation and interference with the activity of efflux pump [35, 36]. The mechanism of their antibacterial effect is also reported by binding to DNA and inhibiting the processes of replication, interacting with

Table 1. Zones of inhibition of isolated pure compounds, Ciprofloxacin and Ketoconazole

	Diameter of Zone of Inhibition (mm) (Mean±SD)				
Sample (100 µg/ disc)	**Bacterial strain**				**Fungal strain**
	Gram-positive		**Gram-negative**		***A. niger***
	B. megatorium	***S. aureus***	***S. typhi***	***E. coli***	
Anhydrofusarubin	16.1±0.5	9.3±0.4	12±1.0	13.3±0.2	--
Fusarubin	25.2±1.0	13.1±0.5	20.2±0.4	20.4±0.3	--
Javanicin	10.4±1.0	10±0.8	8.3±0.8	11±0.2	--
5-deoxybostrycoidin	9±0.3	7.2±0.4	10±0.3	18.4±1.0	--
Ciprofloxacin(30 µg/ disc)	36.1±1.0	30.4±0.5	31.6±1.0	30.3±0.2	nd
Ketokonazole(30 µg/ disc)	nd	nd	nd	nd	25.1±0.5

'—' indicates no activity; 'nd' indicates not done

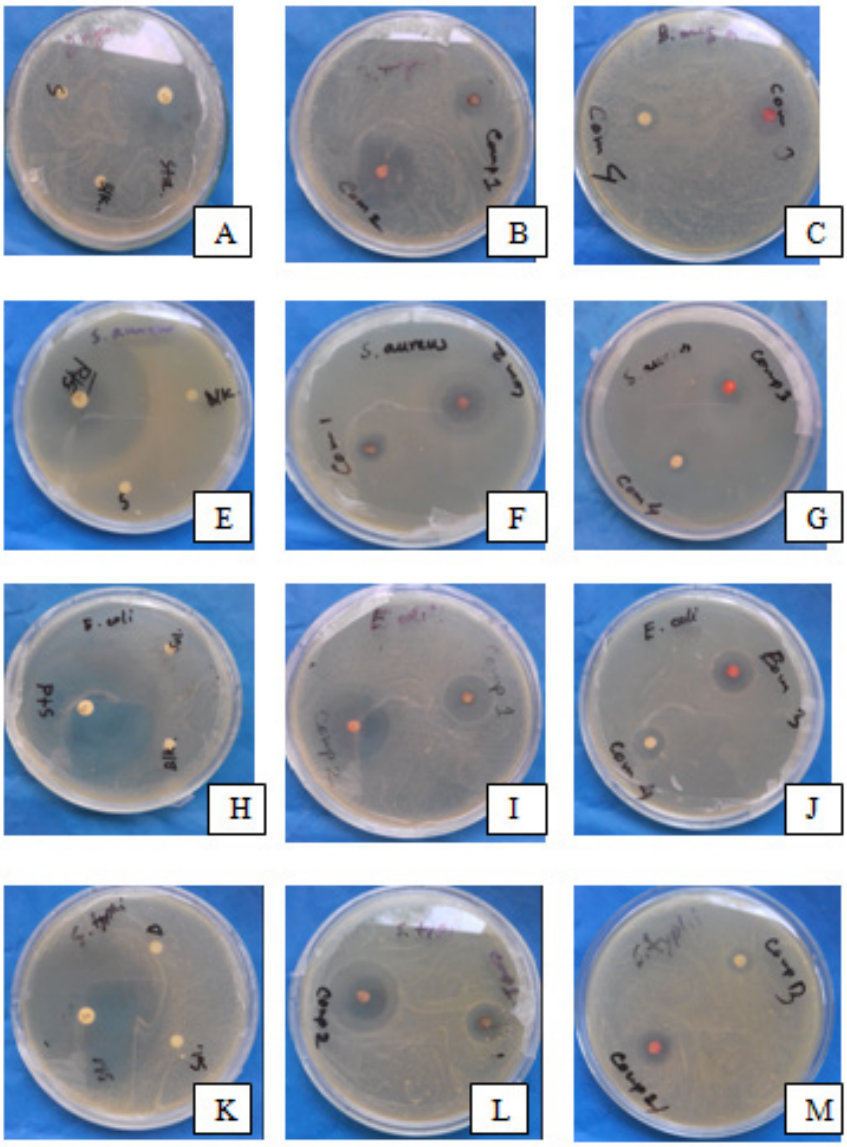

Figure 3. Antibacterial activity of positive control (Ciprofloxacin) and isolated compounds 1-4 against *Bacillus megaterium* (A-C), *Staphylococcus aureus* (E-G), *Escherichia coli* (H-J) and *Salmonella typhi* (K-M)

numerous proteins (enzymes) and disturbing cell and mitochondrial membranes, interfering with electrons of the respiratory chain on mitochondrial membranes [37]. Further study is required to determine the mechanism of action of antibacteria activity of isolated compounds.

Cytotoxicity test

The cytotoxic potential of isolated pure compounds was determined by observing antiproliferative activity on the Human Lung cancer cell line. Trypan blue exclusion method was used to identify the dead cells or viable cells. The result was expressed as the percent dead cell. Doses were given with varying concentrations of the compounds (1, 5, 15 and 20.0 µg/mL). Negative control corresponds to the cells incubated with a medium of 0.6% DMSO.

Vehicle groups (cells cultured with DMSO) were compared with treatment groups (doses at different concentrations). To determine the degree of statistical significance of continuous

variables between groups, they were compared with one-way analysis of variance with post hoc Tukey's test for the analysis of the cytotoxicity of isolated pure compounds (Figure 4). Statistical significance was accepted when $P < 0.001$.

The half maximal value of inhibitory (IC50) activity of each compound was calculated by analysis of the dose-response curve (Figure 5) and summarized in Table 2.

Compound 1 Anhydrofusarubin exhibited significant cytotoxic activity as it shows the highest antiproliferative (% dead cell was 45.70 at 20.0 µg/mL of concentration) activity at different concentrations where % dead cell was increased with increasing concentration & its IC_{50} value was 24.18 µg/mL. The percentage

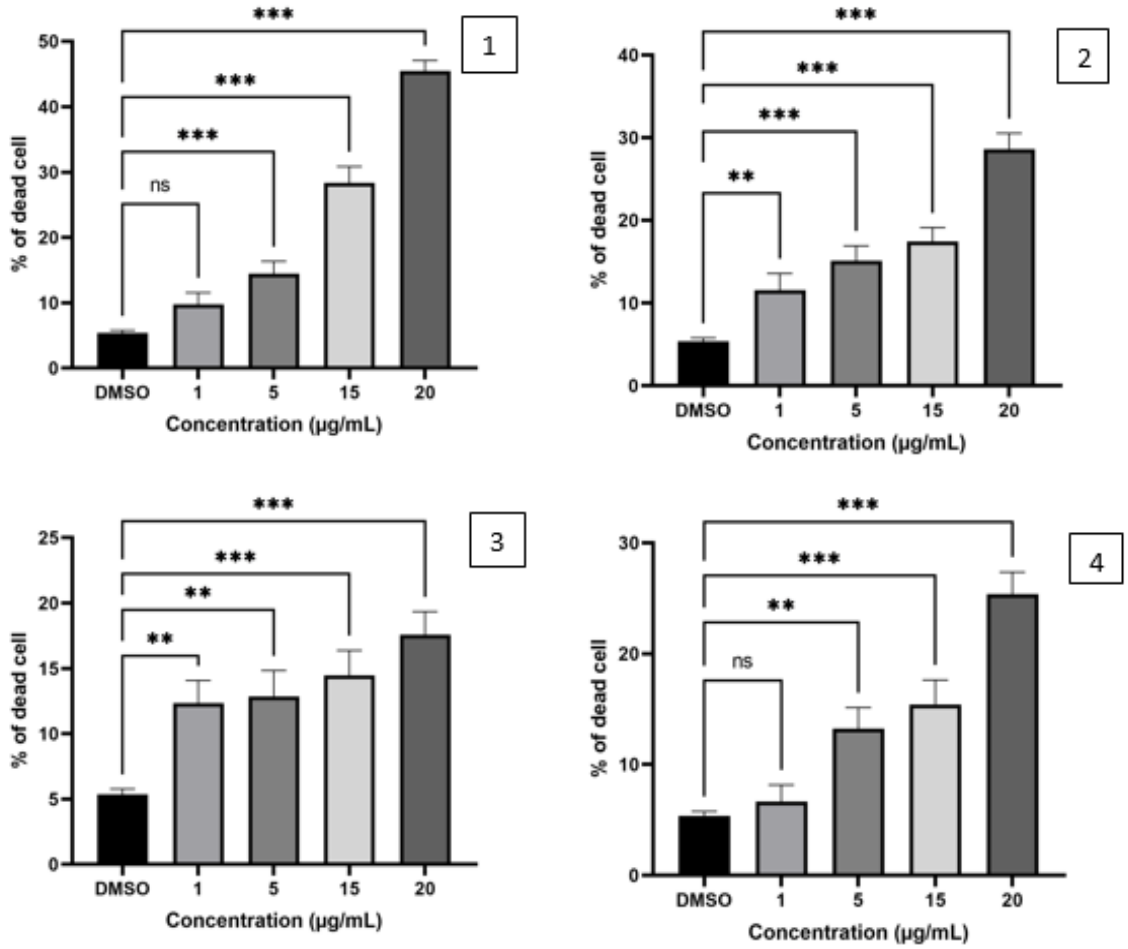

Figure 4. Cytotoxicity effect of isolated compounds on human Lung cancer cell line. Results are compared with vehicle (DMSO). Results are expressed as mean ± SEM (n=3). Degrees of significance were measured using ANOVA with Post Hoc Tukey's test for comparison of isolated compounds with vehicle (P values < 0.001(***); 0.002 (**); 0.033 (*); 0.12 (ns))

Table 2: IC_{50} values of isolated compounds against human Lung Cancer cell line

Compounds	IC_{50} (µg/mL)
Anhydrofusarubin (**1**)	24.18
Fusarubin (**2**)	47.65
Javanicin (**3**)	120.43
5-deoxybostrycoidin (**4**)	52.83

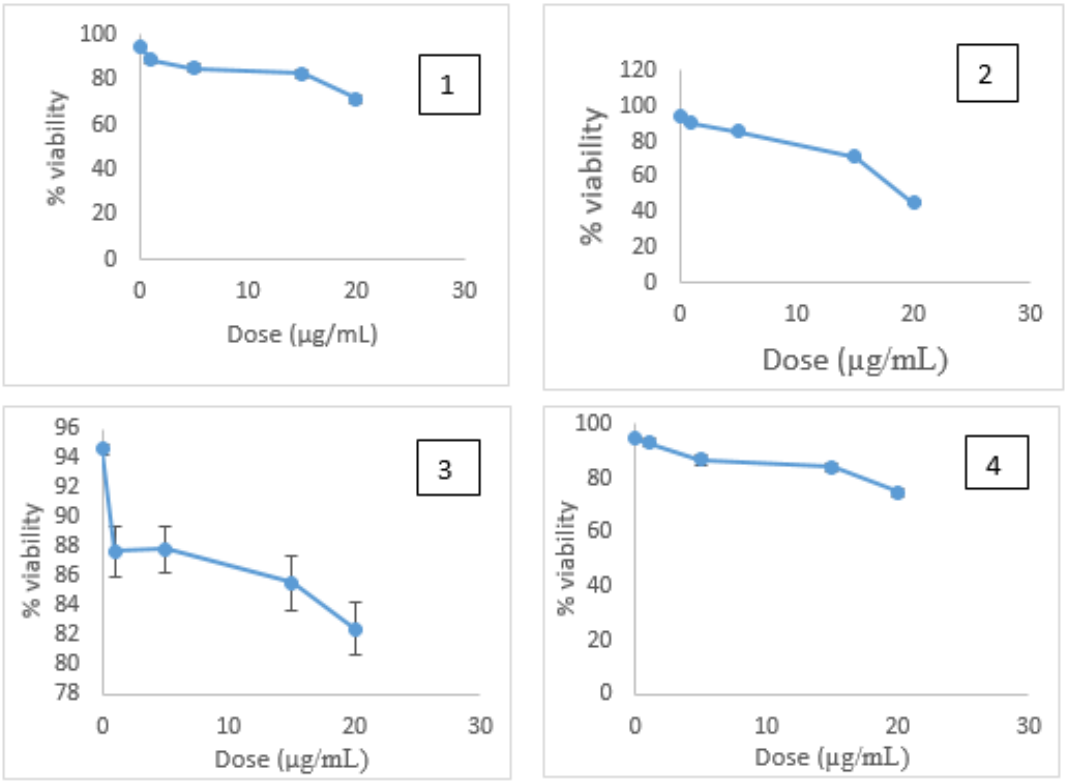

Figure 5. Dose-response curves for the effects of isolated compounds on Lung Cancer cells. The X-axis represents the concentration of samples and the y-axis represents the percentage of cell viability

of the dead cell was nearly 28.97 in Fusarubin (Compound 2), 17.83 in Javanicin (Compound 3) and 25.43 in 5-deoxybostrycoidin (Compound 4) at a concentration level of 20.0 µg/mL. The percentage of dead cells was also increased with increasing concentration for each isolated compound. Fusarubin and 5-deoxybostrycoidin also showed significant cytotoxic activity with Inhibitory Concentration (IC_{50}) values of 47.65 µg/ml & 52.83 µg/ml respectively. Javanicin showed very mild cytotoxic activity as it shows an IC_{50} value of more than 100 µg/mL.

These results indicate that all compounds have cytotoxic activity and may inhibit cell proliferation activity, and activate apoptosis or necrosis in human lung cancer cells. The basic mechanisms of cancer development are abnormal cell growth or cell proliferation, continuous self-renewal and migration of cells with an uncontrolled cell cycle. So these antiproliferative activities would be beneficial to introduce anticancer drugs to lung cancer patients. To the best of our knowledge, this is the first report of the cytotoxic activity of isolated compounds against the Human Lung Cancer Cell Line. The isolated compounds also possess potential antibacterial activity which may be beneficial to develop a drug for chemotherapy-induced bacterial infection in neutropenia. Fusarubin, Anhydrofusarubin and Javanicin are reported to exert insignificant harmful effect on Vero cell (African Green monkey Kidney Fibroblast cell) indicating their (Isolated compounds) specificity to cancer cells [38,39].

Naphthoquinone and aza-antharaquinone either natural or synthetic have earned much attention in the scientific community for their potential pharmacological properties; anticancer property is one of them. Different mechanism of action has been reported for Naphthoquinone and Azaantharaquinone which is also dependent on the variation of chemical structure structure [20,21,40,41]. In our study, three Napthaquinone and one Azaanthraquinone have been isolated with varying

cytotoxic activity. To elucidate the mechanism of action and determine the variation of bioactivity with structural diversity, further study is required.

Conclusion

In a continuation of exploring bioactive compounds from endophytic fungi, three known naphthoquinone (Fusarubin, Anhydrofusarubin, Javanicin) and one known azaanthraquinone (5-deoxy Bostrycoidin) have been reported from endophyte *Fusarium solani.* Isolated compounds possess potential antibacterial activity along with significant cytotoxic activity, highlighting their potential as a lead compound to develop antibacterial and anticancer drugs including antibacterial drugs for cancer-induced neutropenia.

Conflict of interest

Authors have declared that they have nothing to disclose.

Compliance with ethical standard

This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgement

The authors would like to thank the authority of Bangladesh Council of Scientific and Industrial Research (BCSIR) for providing necessary chemicals, reagents and laboratory facilities for this research.

References

1. **Rajaraman, R., Guernsey, D.L., Rajaraman, M.M., Rajaraman, S.R. (2006)**. Stem cells, senescence, neosis and self-renewal in cancer. Cancer Cell International. 6: 25.
2. **Gao, Y., Shang, Q., Li, W., Guo, W., Stojadinovic, A., Mannion, C., Man, Yg., Chen, T. (2020)**. Antibiotics for cancer treatment: A double-edged sword. Journal of Cancer. 11(17): 5135-5149.
3. **Kanady, K.E., Shipley, W.U., Zietman, A.L., Kaufman, D.S., Althausen, A.F., Heney, N.M. (1997).** Treatment strategies using transurethral surgery, chemotherapy, and radiation therapy with selection that safely allows bladder conservation for invasive bladder cancer. Seminars in Surgical Oncology. 13(5): 359-64.
4. **Beyar-Katz, O., Dickstein, Y., Borok, S., Vidal, L., Leibovici, L., Paul, M. (2017).** Empirical antibiotics targeting gram-positive bacteria for the treatment of febrile neutropenic patients with cancer. Cochrane Database Systemetic Review. 6(6): CD003914.
5. **Gafter-Gvili, A., Fraser, A., Paul, M., Vidal, L., Lawrie, T.A., van de Wetering M.D., Kremer, L.C., Leibovici, L. (2012).** Antibiotic prophylaxis for bacterial infections in afebrile neutropenic patients following chemotherapy. The Cochrane Database of Systemetic Review. 1(1): CD004386.
6. 6. **Jackson, N., Czaplewski, L., Piddock, L.J.V. (2018).** Discovery and development of new antibacterial drugs: learning from experience? Journal of Antimicrobial Chemotherapy. 73(6): 1452-1459.
7. **Elghaffar, R.Y.A., Amin, B.H., Hashem, A.H., Sehim, A.E. (2022).** Promising Endophytic *Alternaria alternata* from Leaves of *Ziziphus spina-christi*: Phytochemical Analyses, Antimicrobial and Antioxidant Activities. Applied Biochemistry and Biotechnology. 194: 3984-4001.
8. **Abdelaziz, A.M., El-Wakil, D.A., Attia, M.S., Ali, O.M., AbdElgawad, H., Hashem, A.H. (2022).** Inhibition of *Aspergillus flavus* Growth and Aflatoxin Production in *Zea mays* L. Using Endophytic *Aspergillus fumigatus*. Journal of Fungi. 8: 482.
9. **Hashem, A.H., Shehabeldine, A.M., Abdelaziz, A.M., Amin, B.H., Sharaf, M.H. (2022).** Antifungal Activity of Endophytic Aspergillus terreus Extract Against Some Fungi Causing Mucormycosis: Ultrastructural Study. Applied Biochemistry and Biotechnology. 194: 3468-3482.
10. **Sharaf, M.H., Abdelaziz, A.M., Kalaba, M.H., Radwan, A.A., Hashem, A.H. (2022).** Antimicrobial, Antioxidant, Cytotoxic Activities and Phytochemical

Analysis of Fungal Endophytes Isolated from *Ocimum Basilicum*. Applied Biochemistry and Biotechnology**.** 194: 1271-1289.

11. **Badawy, A.A., Alotaibi, M.O., Abdelaziz, A.M., Osman, M.S., Khalil, A.M.A., Saleh, A.M., Mohammed, A.E., Hashem, A.H. (2021).** Enhancement of Seawater Stress Tolerance in Barley by the Endophytic Fungus *Aspergillus ochraceus*. Metabolites. 11: 428.
12. **Sushanto, G., Gitishree, D., Sen, S.K., Han-Seung, S., Kumar, P.J. (2016).** Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. Frontiers in Microbiology. 7: 1-8.
13. **Zhou, J., Feng, Z., Zhang, W., Xu, J. (2022).** Evaluation of the antimicrobial and cytotoxic potential of endophytic fungi extracts from mangrove plants *Rhizophora stylosa* and *R. mucronata*. Scientific Reports. 12: 2733.
14. **Mohamed, H.F. (2012).** Molecular analysis and anticancer properties of two identified isolates, Fusariumsolani and Emericellanidulans isolated from Wady El-Natron soil in Egypt against Caco-2 (ATCC) cell line. Asian Pacific Journal of Tropical Biomedicine. 2(11): 863-9.
15. **Cai, L., Wang, J., Li, Y., Qin, M., Yin, X., He, Z., Kang, J. (2022).** Global regulatory factor FsveA up-regulate the production of antitumor substance in endophytic *Fusarium solani.* Research Square. DOI: 10.21203/rs.3.rs-1414066/v1.
16. **Wen, J., Okyere, S.K., Wang, S., Wang, J., Xie, L., Ran, Y., Hu, Y. (2022).** Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. Journal of Fungi (Basel). 8(2): 205.
17. **Chowdhury, N.H., Sohrab, M.H., Rony, S.R., Sharmin, S., Begum, M.N., Rana, M.S., Hasan., C.M. (2016).** Identification and bioactive potential of endophytic fungi from *Monochoria hastate* (L.). Bangladesh Journal of Botany. 45(1): 187-193.
18. **Cappiccino, J.G., Sherman, N. (1996).** Microbiology a Laboratory Manual, 6th ed., The Benjamin/ Cummings Publishing Company, Redwood City. CA, USA.
19. **Alzoreky, N.S., Nakahara, K. (2003).** Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology. 80(3): 223-230.
20. **Chowdhury, N.S., Sohrab, M.H., Rana, M.S., Hasan, C.M., Jamshidi, S., Rahman, K.M. (2017).** Cytotoxic Naphthoquinone and Azaanthraquinone Derivatives from an Endophytic *Fusarium solani.* Journal of Natural Products. 80(4): 1173-1177.
21. **Khan, N., Afroz, F., Begum, M., Rony, S.R., Sharmin, S., Moni, F., Hasan, C.M., Shaha K., Sohrab M.H. (2018).** Endophytic *Fusarium solani*: A rich source of cytotoxic and antimicrobial napthaquinone and aza-anthraquinone derivatives. Toxicology Reports. 5: 970-976.
22. **Robert, A.B., James, H.T., Stanly, N.J. (1981).** Toxin Production from *Fusarium solani* from fibrus roots of blight-diseased citrus. Journal of Physiology and Biochemistry. 71: 951-954.
23. **Parisot, D., Devys, M. (1989).**Notizen: 5-Deoxybostrycoidin, a New Metabolite Produced by the Fungus *Nectria haematococca* (Berk. and Br.) Wr.Barbier. ZeitschriftfürNaturforschung B. 44(11): 1473-1474.
24. **Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966).** Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 45(4): 493-6.
25. **Strober, W. (2015).** Trypan Blue Exclusion Test of Cell Viability. Current Protocols in Immunology. 2: 111.
26. **Wilson, C.H., Ali, E.S., Scrimgeour, N., Martin, A.M., Hua, J., Tallis, G.A., Rychkov, G.Y., Barritt, G.J. (2015).** Steatosis inhibits liver cell store-operated Ca^{2+} entry and reduces ER Ca^{2+} through a protein kinase C-dependent mechanism. Biochemical Journal. 466(2): 379-90.
27. **Berry, M.N., Barritt, G.J., Edwards, A.M.**

(1991). Isolated Hepatocytes: Preparation, Properties and Applications, 1st edition, Elsevier (1991).

28. **Hoque, N., Hasan, C.M., Rana, M.S., Varsha, A., Sohrab, M.H., Rahman, K.M. (2018).** Fusaproliferin, a Fungal Mycotoxin, Shows Cytotoxicity against Pancreatic Cancer Cell Lines. Molecules. 23(12): 3288.
29. **Halicki, P.C.B., Ferreira, L.A., De Moura, K.C.G.,Carneiro, P.F., Del Rio, K.P., Carvalho, T.D.S.C., Pinto, M.D.C.F.R., da Silva, P.E.A., Ramos, D.F. (2018).** Naphthoquinone Derivatives as Scaffold to Develop New Drugs for Tuberculosis Treatment. Frontiers in Microbiology. 9: 673.
30. **Koyama, J., Morita, I., Kobayashi, N., Osakai, T., Usuki, Y., Taniguchi, M. (2005).** Structure–activity relations of azafluorenone and azaanthraquinone as antimicrobial compounds. Bioorganic & Medicinal Chemistry Letters. 15(4): 1079-1082.
31. **Kumar, K.P., Javvaji, K., Poornachandra, Y., Allanki, A.D., Misra, S. (2017).** Antimicrobial, Anti-plasmodial and Cytotoxicity Properties of Bioactive Compounds from *Fusarium* sp. USNPF102. Journal of Microbiology Research. 7(2): 23-30.
32. **Baker, R.A., Tatum, J.H., Stan Nemec Jr., S. (1990).** Antimicrobial activity of naphthoquinones from Fusaria. Mycopathologia. 111: 9-15.
33. **Ammar, M.S., Gerber, N.N., McDaniel, L.E. (1979).** New antibiotic pigments related to fusarubin from *Fusarium solani* (Mart.) Sacc. I. Fermentation, isolation, and antimicrobial activities. Journal of Antibiotics. 32(7): 679-84.
34. **Marumo, H., Kitaura, K., Morimoto, M., Tanaka, H., Omura, S. (1980).** The mode of action of nanaomycin A in Gram-positive bacteria. Journal of Antibiotics. 33(8): 885-890
35. **Mone, N.S., Bhagwat, S.A., Sharma, D., Chaskar, M., Patil, R.H., Zamboni, P., Nawani, N.N. and Satpute, S.K. (2021).** Naphthoquinones and their derivatives:emerging trends in combating microbial pathogens. Coatings. 11: 434.
36. **Ravichandiran, P., Sheet, S., Premnath, D., Kim, A.R., Yoo, D.J. (2019).** 1,4-Naphthoquinone analogues:potent anti-bacterial agents and mode of action evaluation. Molecules. 24: 1437.
37. **Babula, P., Adam, V., Havel, L., Kizek, R. (2007).** Naphthoquinones and their pharmacological properties. Ceska a Slovenska Farmacie. 56(3): 114-20.
38. **Vijitphan, P. (2019).** *S*ynthesis of 8-0-Methylfusarubin, 8-0-Methyl anhydro-fusarubin, Fusarubin and Anhydro-fusarubin (Doctoral dissertation, Prince of Songkla University).
39. **Kornsakulkarn, J., Dolsophon, K., Boonyuen, N., Boonruangprapa, T., Rachtawee, P., Prabpai, S., Kongsaeree, P., Thongpanchang, C. (2011).** Dihydronaphthalenones from endophytic fungus *Fusarium* sp. BCC14842. Tetrahedron. 67(39): 7540-7547.
40. **Pereyra, C.E., Dantas, R.F., Ferreira, S.B., Gomes, L.P., Silva-Jr., F.P. (2019).** The diverse mechanisms and anticancer potential of naphthoquinones. Cancer Cell International. 19: 207.
41. **Wellington, K.W. (2015).** Understanding cancer and the anticancer activities of naphthoquinones -a review. RSC Advances. 5: 20309-20338.