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Bioactive Secondary Metabolites from Endophytic *Aspergillus terreus* AH1 Isolated from *Ipomoea carnea* Growing in Egypt

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Abstract

Growing evidence indicates that the endophytic fungus *Aspergillus* is one of rich sources of natural products with a broad spectrum of biological activities. In our effort to isolate and to identify biologically active metabolites from naturally occurring sources, two endophytic fungi have been isolated from the tissues of *Ipomoea carnea*. Among the two isolates, one fungus displayed pronounced antimicrobial activity, against pathogenic microbes includes Gram+ve bacteria; *Staphylococcus aureus* and *Bacillus subtilis*, Gram–ve bacteria; *Pseudomonas aeruginosa* and *Escherichia coli* and Yeasts; *Candida albicans* with inhibition zone range from 16 to 19 mm. The most potent isolate has been identified genetically by sequencing of 18S rRNA gene as *Aspergillus terreus* AH1. Bioguided-fractionation and isolation of *Aspergillus terreus* AH1 extract led to identification of five compounds namely; (+)-asterrelenin (1), (±)-periplanamide B (2), butyrolactone I (3), pyranterrone D (4), and Arenarin A (5). The identified compounds showed a strong antibiofilm activity against human pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Moreover, the compound ((±)-periplanamide B) has strong activity against some carcinoma cell lines such as HCT116, HePG 2 and MCF7

Keywords: *Ipomoea carnea*, Endophytes, *Aspergillus terreus*, secondary metabolites, antimicrobial, antibiofilm, anticancer

1. Introduction

Ipomoea carnea is a perennial shrub that belongs to Convolvulaceae family [1]. Because of its luxuriant vegetative growth and attractive large pink flowers, it was introduced to Egypt as an ornamental plant [2]. *I. carnea* grows on terrestrial land up to a height of 6 m. The stem is dense after a year's growth and develops into a dense trunk and has many thick branches from the base. *I. carnea's* stem is straight, hairy, woody, and of a shape that is cylindrical and greenish. Plant also contains alternate leaves. The leaves are plain and petiolate. Petiole has a cylindrical length of 4.0-7.5 cm and a diameter of 2.5-3.0 mm [3]. Endophytic fungi colonize healthy plant tissues without producing noticeable negative symptoms [4]. Endophytic fungi are associated with medicinal plants and have proven to be an important source for the pharmaceutical industry of different secondary metabolites and bioactive compounds [5,6].

Endophytic fungi penetrated through the stomata along the epidermal cells and colonization in the shoots of plants. On the other hand, the pathogen penetrated directly through the cell wall and extracellularly colonized. Such distinctions between endophytes and pathogenic fungi may not be capable of causing disease in the host plant. Endophytic fungi are documented to help the growth and development of the host plant as well as plant physiology **[7,8]**.

Endophytic fungi obtain from their related host plants nutrients, defense, and replication opportunities and often, host plants benefit from this symbiosis, too **[9]**. Several reports highlighted the importance of endophytes for plants. Theses

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endophytes act as a defense system for the host plants via releasing of wide range of bioactive metabolites such as alkaloids which act as inhibitors for insects and ants. It also reduces microbial infections and induces drought tolerance of host plant **[10]**. The herbs infected with endophytic fungi shows better resistance towards higher temperature. Endophytic fungi can stimulate plant growth, increase resistance towards disease causing pathogens, suppress the weed, and increase tolerance to abiotic and biotic stresses **[11]**. Therefore, the aim of this study was to investigate the bioactive compounds of endophyte *Aspergillus terreus* AH1 isolated from *I. carnea* tissues. Furthermore, the antimicrobial, antibiofilm, antioxidant and anticancer activity were also investigated.

2. Materials and Methods

Plant samples collection

Plant samples were collected from polluted soil in Elbehira Governorate, Egypt. The samples were numbered, transported in an icebox to the Ecology Laboratory, and identified by Dr. Albara Salaheldin at Al-Azhar University's Faculty of Science. The identified specimens were sent to the Biotechnology Lab. at Al-Azhar University's Faculty of Science (Girls Branch), Cairo, Egypt.

Chemicals and reagents

Methanol, *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol were purchased from Adwic-El Nasr Pharmaceutical Co. (Cairo, Egypt). Potato dextrose agar was purchased from Merck Chemical Co. (Darmstadt, Germany). All other chemicals and reagents were the highest analytical grade and purchased from common sources.

Endophytic fungal isolation

The plant parts surfaces were sterilised as previously mentioned by Elkhouly *et al*. (2021) **[12]**. After sterilization, the healthy plant part was cut into small pieces measuring 1 cm^2 and put on a PDA plate for approximately 5-6 days to investigate the growth of any endophytic fungi **[12]**.

Identification of endophytic fungus

To identify the selected endophytic fungus, the DNA was extracted using Qiagen DNeasy Mini Kit protocol, amplified using two universal primers ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and ITS4 (5′-TCCTCCGCTTATTG ATATGC-3′) and sequenced by Macrogen Companies, Seoul, South Korea. To identify the homology and similarity of the obtained sequence, it was aligned with known sequences available at gene bank using BLAST tool available online (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using by MEGA 7 program according to Kumar *et al*. (2016) **[13]**. The resulting gene sequence was submitted to the NCBI GenBank database and an accession number was attained.

Extraction, purification and structural elucidation of bioactive compounds

Purification was started with liquid-liquid partitioning, followed Kupchan's protocol **[14]**. Based on chemical and biological screening, the fraction of interest was subsequently purified using Sephadex LH-20. Five compounds were obtained after further purification with semipreparative HPLC. NMR spectroscopy was used to determine the structure of the purified molecule, as well as a Thermo Scientific LTQ Orbitrap XL mass spectrometer to determine the molecular formula.

Antimicrobial activity of crude ethyl acetate extract

The antimicrobial activity for fungus crude extract was performed using agar diffusion method **[15,16]**. The endophyte fungus extract was dissolved in MeOH at 500 µg/ml and Aliquots of 50 µl were loaded on disks (Whattman No. 1 filter paper, 5 mm). The inhibition zone diameter was measured, and various of pathogenic microorganisms comprising Gram+ve bacteria; *Staphylococcus aureus* (ATCC6538-P), *Bacillus subtilis* (ATCC6633), Gram –ve bacteria *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC25955) and yeast; *Candida albicans* (ATCC10231) were observed. Antimicrobial activity for pure compounds was assessed using 96 microplate methods **[17]**. All test microbes were obtained from the Culture Collection Center (Microbial Chemistry Department and National Research Centre, NRC), Egypt.

Biofilm inhibitory activity

The biofilm inhibitory activity of the crude and purified compounds was measured using 96 well-flat

bottom polystyrene titer plates MTP assays toward four clinical microbes *P. aeruginosa*, *S. aureus*, *E. coli* and *B. subtilis*) following the method of Christensen *et al.* (1985) **[18]** and Hamed *et al.* (2020) **[19]**. The optical density was estimated at 570 nm using (Spectrostar nanomicroplate reader (BMG LABTECH GmbH, Allmendgrun, Germany).

***In vitro* cytotoxic activity**

The cell lines used in this study (human lung fibroblast normal cell line (WI38), colorectal carcinoma colon cancer (HCT-116), breast cancer of the mammary gland (MCF-7), and hepatocellular cancer (HePG-2)) were obtained from Biological Products and Vaccine Holding Company (VACSERA), Cairo, Egypt. Doxorubicin has been used as control. The inhibitory effects on the above-mentioned cell lines growth were determined using MTT. The colorimetric assay depends on the conversion of yellow tetrazolium bromide (MTT) in viable cells into a violet formazan derivative by mitochondrial succinate dehydrogenase. The cell lines were cultivated using 10 % fetal bovine serum in the RPMI-1640 medium supplemented with 100 units/ml of penicillin and 100µg / ml of streptomycin. The cells were treated with compounds and incubated for 24 h. A 20 µl of MTT solution at 5 mg/ml was used in the drug treatment, added and incubated for 4 h, and 100µl of DMSO was added to dissolve the purple formazan formed in each well. The colorimetric assay was recorded at 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) x 100 **[20,21]**.

3. Results and Discussion

In the current study, two endophytic fungi have been isolated from plant *Ipomoea carnea* growing in Egypt (El Beheira Governorate) as recorded in table (1). Endophytes can actively or passively promote plant growth, providing a variety of host fitness, increased plant resistance to abiotic and biotic stresses through a variety of mechanisms **[22]**. Tayung *et al.* (2012) isolated 69 isolates belonging to ten taxa including 3 isolates of sterile mycelia and 2 unidentified species were obtained from the leaves, barks, and seeds of *I. carnea* **[23]**.

Table (1): Isolated endophytes from *I.carnea* plant.

plant	Isolate code	Location of plant	Color of endophyte
I.carnea	DBF1	El-Beheira-Egypt	Brown
	DBF5		Brown

Evaluation of the antimicrobial activities of endophytic microorganisms

The crude extracts prepared from 2 endophytic microorganisms were evaluated (*in vitro*) for their antimicrobial activity against tested pathogenic microorganisms; two-Gram negative bacteria, two-Gram positive bacteria and one yeast as recorded in table (2).The results showed that out of 2 endophytic microorganisms only one isolate exhibited antimicrobial activity against all tested pathogenic microorganisms the most suitable microbe was *candida albicans* with inhibition zone 19 mm approximately high as the control, as recorded in table (2).

Asfour *et al*. (2019) **[24]** reported that, the antimicrobial assay of crude *A. terreus* extracts toward four pathogenic microbes: *B. subtilis*, *P. aeruginosa*, *S. aureus* and *C. albicans*. The test was carried out using 50 µL of the extract (2 mg/mL, DMSO).

Tables (2). Biological activity of some isolated endophytic fungi against some pathogenic microorganisms

Tested pathogens	Fungal isolates		
	DBF1	DBF5	Standard
Yeast	Diameter (mm) ± SD		Ampho
C. albicans	0±0.0	19±0.02	20±002
Gram+ve			Vanco
S. aureus	0±0.0	16±0.197	20±031
			Ampi
B. subtilis	0±0.0	17.5±0.25	21±0.05
Gram-ve			Genta
P. aeruginosa	0±0.0	16.5±0.21	20±0.05
E. coli	0±0.0	17±0.21	20±0.15

Ampho: Amphotericin Vanco: Vancomycin Ampi: Ampicillin Genta: Gentamicin

The results showed that, the extract showed antimicrobial activity toward *aeruginosa* and *S. aureus* with inhibition zones ranged from 15-7mm

and no activity detected against *B. subtilis* and *C. albicans*.

Tayung *et al.* (2012) found that out of 69 endophyte isolates, only 15 isolates (21.74%) displayed antimicrobial activity against the tested microorganisms either against bacteria or fungi **[23]**. Phongpaichit *et al*. (2006) obtained 70 endophytic fungal stains from five medicinal *Garcinia* plants and the majority of them showed antimicrobial activity against *Candida albicans, Staphylococcus aureus, Cryptococcus neoformans and Microsporum gypseu* **[25]**. Konings *et al.* (1992) investigated that, extracts of fungal endophyte have antimicrobial activity because of different fungal strains could secrete intracellular and extracellular bioactive metabolic products. The efficacy of these extracts against *P. aeruginosa*, *S. aureus*, *B. subtilis* and *C. albicans* might have the potential to serve as drug leads to treat a wide variety of diseases **[26]**.

Antibiofilm against some pathogenic microorganisms

Biofilms are complex structures formed from an extracellular polymeric substance (EPS) composed from protein, polysaccharides, and DNA **[27]**. This structure enables the bacteria to attach on living and non-living objects **[28]**. In bacterial biofilm, the EPS structure protect the bacteria from antibiotics by reducing the penetration of antibiotics. Almost, 80 % of the bacterial biofilm led to multidrug resistant (MDR) effect in bacteria **[29]**. Both Gram negative (GN) and Gram positive (GP) bacteria form biofilms including *Escherichia coli, Staphylococcus aureus, P. aeruginosa, Bacillus subtilis, and K. pneumonia* **[30]**. They may be developed in the tissue of the patient or on the surface of human medical equipment such catheters, laryngeal tubes or stents **[31]**. Different strategies have been developed for the efficient biofilm control **[32,33]**, however, new effective anti-biofilm agents to inhibit the biofilm formation are still highly needed. **[34,35]**. From the results we can find that, isolate DBF5 extract is an active endophyte extract which inhibit the formation of bacterial biofilm.

The evaporated ethyl acetate extract was diluted with methanol for MTP test that was performed according to Christensen *et al*. (1985) **[18]**. From the DBF5 extract, a concentration (100 µg/mL) was selected for biofilm inhibition assay against tested pathogens and their virulence factor in terms of biofilm formation was performed in a 69-well microtiter plate. The crude extract from fungal endophyte DBF5 showed better inhibition percentage in (100 µg/mL) with 88.15% against *Pseudomonas aeruginosa*, 59.04% against *Staphylococcus aureus*, 44.7% against *Bacillus subtilis* and 19.05% against *E coli*. The results showed that the crude compound of DBF5 was the most active against biofilm colonization and cell adherence as shown in table (3).

Table (3): Biofilm inhibition of crude endophyte extract against pathogenic bacteria

Extract	Antibiofilm formation %			
	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*
DBF5	19.05±2.02	59.04±6.02	44.7±8.02	88.15±2.02

Identification of the most active isolates

Molecular Identification

The sequence of 18S rRNA gene was obtained, identified and aligned against other identified sequences available in the GeneBank database using BLAST tool to identify the similarity score and to calculate the statistical significance of the matches (http://www.blast.ncbi.nlm.nih.gov/Blast). The obtained result confirmed a very close similarity of the 18S rRNA gene sequence with 100 % homology of the isolate DBF5 with *Aspergillus terreus*. The phylogenetic analysis and tree were constructed using the neighbour-joining method (Fig. 1) by MEGA 7 program according to Kumar *et al*. (2016) **[13]**. Based on the analysis of the DNA sequence and the morphological characteristics of the s DBF5 isolate was identified as *Aspergillus terreus* AH1 and deposited in GenBank with the accession no. MN784506.1

Structural elucidation of the isolated compounds

The isolate *Aspergillus terreus* AH1 was cultivated on rice medium and extracted with ethyl acetate. The ethyl acetate evaporated using rotary evaporator till complete evaporation of ethyl acetate. The extract (14.3g), was primarily fractionated using Liquid-Liquid partitioning into five fractions. Based on the biological screening, the most active fraction (Dichloromethane) was further purified using

Sephadex LH-20 column with a gradient mobile phase DCM: Methanol. The TLC chromatogram showed that fraction no. (9) contains the peaks of interest. The fraction was further purified using semipreparative HPLC to yield five compounds.

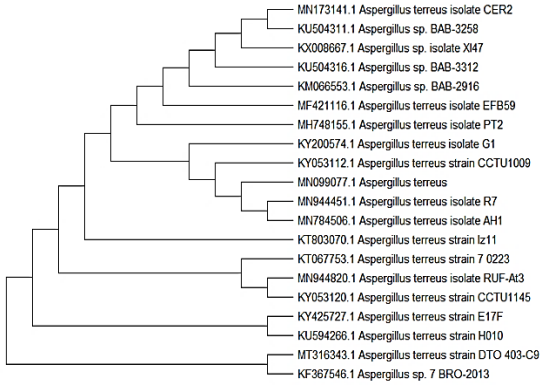


Fig. (1). Constructed phylogenetic tree of *Aspergillus terreus* AH1

Compound 1 ((+)-asterrelenin) (2mg) was obtained as colorless crystals. The ^{1}H NMR showed characteristic signals for olefinic protons at δ_H 5.06 (1H, d, *J*= 10.5 Hz), 5.09 (1H, d, *J*= 17.5 Hz), and 5.90 ppm (1H, dd, *J*= 17.5, 10.5 Hz), and methyl protons at δ_H 0.86 and 1.08 ppm (each 3H, s) suggested the presence of aliphatic moiety [-$C(CH3)_2CHCH_2$]. Also, characteristic signals were resonated between δ_H 6.92 and 7.85 suggested the presence of two ortho-substituted aromatic rings. The aromatic protons of the first phenyl ring were resonated at δ_H 7.85 (1H, dd, *J* = 7.5, 1.5 Hz, H-5), 7.02 (1H, d, *J*= 7.5, 0.85 Hz, H-6), 7.63 (1H, dd, *J*= 6.8, 1.2 Hz, H-7), and 7.42 ppm (1H, d, *J*= 7.5, 1.5 Hz, H-8), while the protons of the second phenyl ring were appeared at δ_H 6.92 (1H, dd, *J*= 8.0, 1.2 Hz, H-18), 7.15 (1H, d, *J*= 8.0, 0.85 Hz, H-19), 7.10 (1H, d, *J*= 7.5, 0.85 Hz, H-20), and 7.35 ppm (1H, dd, *J*= 7.5, 0.85 Hz, H-21). Another set of aliphatic protons including methyl and olefinic signals were resonated at δ_H 6.20 (1H, s, H-2), 5.90 (1H, dd, *J*= 17.5, 10.5 Hz, H-23), 5.1 (1H, d, *J*= 17.5 Hz, H-24a), 5.05 (1H, d, *J*= 10.5 Hz, H-24b), 3.60 (1H, d, *J*= 13.5 Hz, H-10a), 2.61 (3H, s, H-28), 2.39 (1H, d, *J*= 13.5 Hz, H-10b), 1.07 (3H, s, H-25), and 0.90 ppm (3H, s, H-26). On the basis of its HRESIMS (positive ion mode) *m/z* 432.1928 $[M+H]^+$ (calc for $C_{25}H_{25}N_3O_4$, 431.1761) (Figure 1), this compound could be identified as (+)-asterrelenin based on comparing the given spectral data with the available reported data **[36]**.

Compound 2 ((±)-periplanamide B) (923 µg) was obtained as pale-yellow gum. The ^{1}H NMR showed the existence of one ABX system at δ_H: 6.85 (1H, d, J = 2.1 Hz, H-2), 6.74 (1H, d, *J* = 8.1 Hz, H-5), and 6.68 ppm (1H, dd, *J* = 8.1, 2.1 Hz, H-6) were assigned to aromatic nucleus, as well as two oxygenated aliphatic methine protons (-O-CH-) were resonated at δH 4.55 (d, *J* = 4.9 Hz, H-7), and δ_H 5.07 ppm (d, J = 4.9 Hz, H-8), as well as a methoxy group was appeared at δ_H 3.28 ppm (s, 8-OCH_3), and a characteristic N-acetyl methyl group (-NH-CO-CH_3) was resonated at δ_H 1.99 ppm (s, H-10). On the basis of its HRESIMS (positive ion mode) m/z 242.1026 [M+H]+ (calc for $C_{11}H_{15}NO_5$, 241.1026) (Figure 2), this compound could be identified as (±)-periplanamide B based on comparing the given spectral data with the available reported data **[37]**.

Compound 3 (butyrolactone I) (3mg) was obtained as colorless fine crystals. The ^{1}H NMR showed the existence of characteristic aromatic protons for two phenyl rings were appeared at δ_H 7.60 (2H, d, *J* = 8.9 Hz, H-2',6'), 6.90 (2H, d, *J* = 8.9 Hz, H-3',5'), 6.48 (1H, dd, *J* = 7.0, 2.1 Hz, H-6"), 6.47 (1H, d, *J* =8.0 Hz, H-5"), and 6.42 ppm (1H, d, *J* = 1.8, H-2"). Another set of olefinic, methylene and methyl protons were resonated at δ_H 5.02 (1H, t, *J* = 3.8 Hz, H-8"), 3.78 (3H, s, 7-OCH_3), 3.45 (2H, d, *J* = 3.8, CH2-5), 3.09 (2H, d, J = 7.1 Hz, CH2-7"), 1.67 (3H, s, CH3-10"), and 1.57 ppm (3H, s, CH3-11"). Based on its HRESIMS (positive ion mode) *m/z* 425.1596 [M+H]+ (calc for $C_{24}H_{24}O_7$, 424.1596) (Figure 3), this compound could be identified as Butyrolactone I based on comparing the given spectral data with the available reported data **[38]**.

Compound 4 (pyranterrone D) (1.5mg) was obtained as yellow powder. The ^{1}H NMR showed the existence of characteristic olefinic protons were appeared at δ_H 7.40 (1H, dd, *J* = 15.5, 8.3 Hz, H-2), and 6.94 ppm (1H, d, *J* = 15.5, H-3). Another set of aliphatic protons including methine, methylene, and methyl protons were resonated at δ_H 3.87 (1H, br s, H-7), 3.62 (1H, dd, *J* = 11.5, 4.3 Hz, H-8a), 3.58 (1H, dd, *J* = 11.5, 3.1, Hz, H-8b), 6.39 (1H, m, H-9), 6.42 (1H, m, H-10), 2.16 (2H, m, H-11), 1.45 (2H, m, H-12), and 0.91 ppm (3H, t, *J* = 6.4 Hz, H-13). On the basis of its HRESIMS (positive ion mode) *m/z*

252.1239 [M+H]+ (calc for $C_{13}H_{17}NO_4$, 251.1239) (Figure 4), this compound could be identified as Pyranterrone D based on comparing the given spectral data with the available reported data **[39]**.

Compound 5 (Arenarin A) (1mg) was obtained as fine crystals. The ^{1}H NMR showed the existence of characteristic aromatic protons for three phenyl rings were resonated at δ_H 7.48 (1H, s, H-6), 7.05 (1H, s, H-3), 7.15 (2H, d, *J* = 8.35 Hz, H-2"/6"), 6.75 (2H, d, *J*= 8.35 Hz, H-3"/5"), and 7.08 ppm (1H, s, H-6'). Another set including olefinic, methylene, methyl and methoxy signals were resonated at δ_H 5.49 (1H, m, H-8), 4.59 (2H, d, *J* = 6.60 Hz, H-7), 3.77 (3H, s, 3'-OCH_3), 1.77 (1H, s, H3-10), and 1.76 ppm (3H, s, CH_3-11). Based on its HRESIMS (positive ion mode) m/z 407.1492 [M+H]+ (calc for $C_{24}H_{22}O_6$, 406.1492) (Figure 5), this compound could be identified as Arenarin A based on comparing the given spectral data with the available reported data **[40]**.

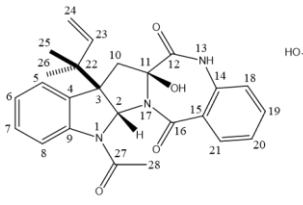
Evaluation of bioactivity by partial purified compounds

First of all, we made large scale fermentation the most potent endophytic fungi by using a rice state fermentation medium, 18-liter medium for the fungus and incubated for 15 days at 30 °C after incubation period we extracted secondary metabolites by ethyl acetate and evaporated using rotary evaporator at 40 °C. The yield production from DBF5 was 14.3 g/18 L medium. Then the bioactive compound was precipitated according to the method of Kupchan original protocol-1 **[14]**. From *A. terreus* AH1 endophyte we get five extractions aqueous methanol, hexane, DCM, butanol. The results showed that both extracts of fungal endophytic DBF5 for methanol and DCM have the most antimicrobial activities against pathogenic microorganisms as shown in table (4).

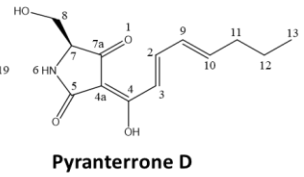
Table (4): Antimicrobial activity of all extracts of DBF5 against some pathogenic microorganisms

Tested pathogens	Fungal isolates					
	DBF5 METH	DBF5 HEXA	DBF5 DCM	DBF5 BUT	DBF5 H2O	*Standard*s
Yeast	Diameter(mm) ±SD					Ampho
C. albicans	18±0.02	0	17±0.02	0	0	20±002
Gram+ ve						Vanco
S. aureus	16±0.15	0	16±0.18	0	0	20±031
						Ampi
B. subtilis	14±0.55	0	14±0.25	0	0	21±0.05
Gram-ve						Genta
P. aeruginosa	15±0.25	0	15±0.27	0	0	20±0.05
E. coli	14±0.09	0	16±0.28	0	0	20±0.15

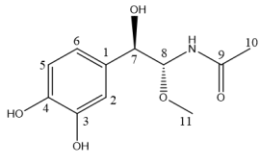
Ampho: Amphotericin Vanco: Vancomycin Ampi: Ampicillin Genta: Gentamicin



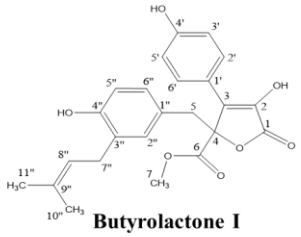
(+)-asterrelenin



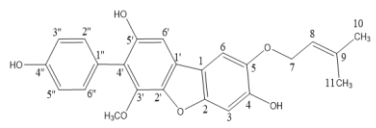
Pyranterrone D



(±)-periplanamide B



Butyrolactone I



Arenarin A

Zerroug *et al.* (2018) studied antimicrobial activity of different crude extracts of *P. griseofulvum* on agar well diffusion method. All the pathogenic bacteria were inhibited by ethyl acetate and dichloromethane crude extracts with the maximal activity showed against *E. coli* ATCC 25922 (45.5 and 41.0 mm respectively), unlike n-hexane crude extract which did not inhibit any pathogenic bacteria **[41]**.

Evaluation of bioactivity of pure isolated compounds

Antibiofilm activity

Data illustrated in table (5) represent the antibiofilm activity of the pure compounds from *Aspergillus terreus* AH1 against a set of microorganisms comprising Gram-ve bacteria and Gram+ve bacteria (*E. coli, S. aureus, B. subtilis* and

P. aeruginosa). The pure extracts from fungal endophyte *A. terreus* AH1 showed better inhibition in 100 µg/mL.

Where (±)-periplanamide showed excellent inhibition with 90.65% against *S. aureus* and 85.09% against *B. subtilis* and very good activity 63.144% against *E. coli.* Also, butyrolactone showed very good inhibition with 71.81% against *P. aeruginosa* as shown figure (3). The result revealed that the pure compounds of *A. terreus* AH1 acted more potent against biofilm colonization and cell adherences.

Table (5): Antibiofilm of some isolated pure compounds from endophyte *A. terreus* AH1

Compounds	Antibiofilm formation %			
	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*
(±)-periplanamide B	63.144	90.65	85.09	59.34
Butyrolactone	42.52	76.38	52.55	71.81

Antimicrobial activity

Antibacterial activity results of pure compounds ((±)- periplanamide B and butyrolactone) that isolated from *A. terreus* AH1 toward pathogenic G+ve and G-ve pathogenic bacteria were recorded in table (6). The results showed that (±)- periplanamide has very strong inhibition activity on growth of *E. coli*, *S. aureus* and *B. subtilis* that gave 0.461, 0.236 and 0.503 O.D. respectively in comparison with control and has moderate activity on *P. aeruginosa* that gave 0.743 O.D. in comparison with control.

Yan *et al.* (2018) extracted three new compounds, periplanamides A (1) and B (2), periplanpyrazine A (3) from the medicinal insect *Periplaneta americana*. These isolates have biological activities toward nitric oxide (NO) production, cell proliferation in HDFs (Human dermal fibroblasts), cell migration and angiogenesis in HUVECs (Human umbilical vein vascular endothelial cells) they have excellent therapeutic effects on wound repair and ulcer **[37]**.

Table (6): *In vitro* antimicrobial activity of (±)-periplanamide B and Butyrolactone isolated from endophyte *A. terrus* against four pathogenic microbial strains

Extract	Antimicrobial activity at O.D (570)			
	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*
(±)-periplanamide B	0.461	0.236	0.503	0.743
Butyrolactone	0.826	0.462	0.697	0.693
Control	1.346	2.005	1.002	1.686

Cytotoxic activity of some compounds against human cell line

The cytotoxic activity of the test extract and pure compounds on MCF-7, HCT-116, and HepG2 carcinoma cells and normal cell (WI38) were tested, and the results are presented in Table 5. The results revealed that, for the pure compound Butyrolactone IC_{50} value of 38.11 µg/well was recognized against the colon carcinoma cells, IC_{50} value of 43.75 µg/well for hepatocellular carcinoma, and IC_{50} value of 33.82 µg/well for breast carcinoma. Also, for the pure compound ((±)-periplanamide B) IC_{50} value of 12.86 µg/well for colon, IC_{50} 15.20 µg/well against hepatocellular carcinoma and IC_{50} 9.58 µg/well against breast carcinoma and for crud extract IC_{50} value of 7.99 µg/well for colon, IC_{50} 8.62 µg/well against hepatocellular carcinoma and IC_{50} 6.91 µg/well against breast carcinoma (Table 7). The results indicated that the crude extracted from *A. terreus* shows significant very strong antiproliferative activity against colon, hepatocellular and breast carcinoma cell line as compared with control (DOX). Also, the pure compound ((±)-periplanamide B) showed strong antiproliferative activity while the pure compound Butyrolactone showed moderate antiproliferative activity against examined cell lines cancer of the colorectal colon (HCT-116), breast cancer of the mammary gland (MCF-7), and (HePG-2).

Table (7): *In vitro* antiproliferative activities (IC_{50}, µg/mL) of isolated endophyte *A. terreus* crude extract and pure compounds against human carcinoma cells (MCF-7, HCT-116, and HepG2). Also, against normal cell line (WI38)

Comp.	*In vitro* Cytotoxicity IC_{50} (µg/ml)•			
	WI38	HCT116	HePG-2	MCF7
Doxorubicin	6.72±0.5	5.23±0.3	4.50±0.2	4.17±0.2
Butyrolactone	52.83±3.2	38.11±2.8	43.75±3.1	33.82±2.6
(±)-periplanamide B	25.49±1.9	12.86±1.1	15.20±1.3	9.58±0.8
Crude extract	20.65±1.6	7.49±0.6	8.62±0.7	6.91±0.5

• IC_{50} (µg/ml) 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)

Asfour *et al*. (2019) showed that, the extracts showed variable antiproliferative activity against the cell lines under investigation. Amongst these, extracts of S004, S006, S016, S017, and S020 presented the most promising antiproliferative profile (IC_{50} values ranged from 23-62 µg/mL). Also, they

reported that, the cytotoxic effect of pure compound terrain against HCT-116 and HepG2cancer cell lines (concentration range 0.01–100 µM) was assessed using the SRB-U assay **[24]**.

Skehan *et al*. (1990) reported that, the compound displayed strong antiproliferative activity against the two cell lines under investigation, with IC_{50} values of 12.13 µM and 22.53 µM for HCT-116 and HepG2 cells, respectively. Doxorubicin was used as a standard cytotoxic control **[42]**.

4. Conclusions

In this study, 2 endophytic fungal strains were isolated from *I. carnea* plant, biological screening including antibacterial and antibiofilm, Bio-activities of two fungal ethyl acetate extracts, showed that, *A.terrus* AH1 exhibited maximum antibacterial activity against tested pathogenic strains with inhibition zone of 19 mm. Moreover, the isolated pure compounds Butyrolactone and (±)-periplanamide B inhibited biofilm formation up to 85.09%. The IC_{50} values of the (±)-periplanamide B compound against some carcinoma cells such as HCT116, HePG 2, and MCF were 12.86, 15.20, and 9.58 µg/well, respectively, described as very strong activities.

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Abbreviations

rRNA: Ribosomal Ribonucleic Acid; HCT116: Human Colon Cancer Cell Line; HePG 2: Human Liver Cancer Cell Line.; MCF7: Breast Cancer Cell Line; PDA: Potato Dextrose Agar; DNA: Deoxyribonucleic Acid; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; HPLC: High Performance Liquid Chromatography; NMR: Nuclear Magnetic Resonance; ATCC: American Type Culture Collection; WI38: Human Lung Fibroblast Normal Cell Line; DMSO: Dimethyl Sulfoxide; Gram+ve: Gram-Positive; Gram-ve: Gram-Negative; MDR: Multidrug Resistant; DCM: Dichloromethane; TLC: Thin Layer Chromatography; *J*: Coupling Constant; Hz: Hertz; ppm: Parts Per Million; HRESIMS: High Resolution Electrospray Ionization Mass Spectrometry; O.D.: Optical Density; HUVECs: Human Umbilical Vein Vascular Endothelial Cells; HDFs: Human Dermal Fibroblasts; IC_{50}: Half Maximal Inhibitory Concentration; DOX: Doxorubicin.

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