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GC-MS, antibacterial, antibiofilm, and anticancer properties against MCF–7 and HEPG2 cell lines of *Aspergillus terreus* AUMC 15762 crude extract

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ABSTRACT

In this investigation, GC-MS analysis was used to assess the chemical composition of the aqueous crude extract generated by *A. terreus* AUMC 15762 in a liquid medium. There were twenty-five identified chemical components in the crude extract, including fatty acids, monosaccharide derivatives, tertiary amines, alkaloids, ethylbenzenes, polyethylene oxides, Phenolic derivatives, furan derivatives, pyranones derivatives, oxygenated hydrocarbons, and halogenated alcohol. *A. terreus* crude extract at 20, 10, 5, and 2.5 mg/mL has demonstrated suppression of *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, respectively. Inhibition zones against the bacterial strains were shown to be 20±1, 18±2, 19±2, and 20±1 mm at 2.5 mg/mL. Antibiofilm capability was demonstrated by 92.69, 92.00, 93.46, and 88.17 %, against *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis*, respectively. Moreover, the crude extract of *A. terreus* has exhibited a noteworthy cytotoxic reaction against the human breast carcinoma cells (MCF–7) and human hepatocarcinoma cell lines (HepG2), with IC_{50} values of 156.84±2.15 µg/mL and 120.59±1.96 µg/mL, respectively. The objective of this research is to examine the chemical profile that was extracted using aqueous from *A. terreus* AUMC 15762 and determine its antibacterial, antibiofilm, and anticancer properties.

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Introduction

A large class of low molecular weight organic molecules known as secondary metabolites (SMs) are derived from both basic and inorganic substances (Erb and Kliebenstein 2020). SMs were created by diverse spectra of plants, animals, and microbes. One typical source of bioactive SMs is fungi. By learning to tolerate a range of environmental stresses and risky circumstances, they may boost their chances of survival even though they may not directly contribute to growth and development or be required for the growth of their producer (Erb and Kliebenstein 2020, Newman and Cragg 2020a, b).

Numerous secondary metabolites (SMs), including vitamins, pigments, amino acids, cytotoxic, immunosuppressive, antibiotics, and other organic compounds, are known to be produced by fungi. These

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SMs are important for applications in medicine, pharmaceuticals, and the food industry because they have biological activities recognized (Devi et al. 2020, Kourkoutas et al. 2015, Natthapat 2018).

Although the Species Fungorum database currently lists over 161,000 fungal species (https://www.speciesfungorum.org/Names/Names.asp), the identification of new secondary metabolites (SMs) has proven difficult, leading to the identification of only a small number of fungal secondary metabolites to date (Hawksworth and Lücking 2017). The most active fungi are species of *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium*, and *Penicillium*, which together create about 15,600 fungal metabolites (Sulyok et al. 2020).

About 500,000 secondary metabolites—also known as natural products—have been published. Of them, 350,000 come from plants, 100,000 from animals, and 70,000 are derived from microorganisms (Bérdy 2012, Bills and Gloer 2016, Nett et al. 2009, Abo Nahas et al. 2023). There are already over 33,500 known bioactive microbial metabolites (Nett et al. 2009). Of these 33,500 microbial metabolites, approximately 41% (13,700) are products of Actinomycete fermentations, approximately 47% (15,600) are derived from fungi, and approximately 12.5% (4,200) are metabolites of unicellular bacteria and cyanobacteria (Bérdy 2012).

On the other hand, research on fungal metabolites has led to the discovery of numerous significant medications. Natural products have been crucial in the development of successful treatments for cancer, malaria, bacterial and fungal infections, neurological and cardiovascular diseases, and autoimmune disorders. They remain one of the most significant therapeutic agents and lead compounds in medicine (Newman and Cragg 2016).

Bacteria are so common that they often cause infections that lead to the development of human diseases. These infections promote the growth of bacterial pneumonia, typhoid fever, acute gastroenteritis, diarrhea, and periodontal disease in susceptible hosts. Antibiotics or antimicrobial therapy may be utilized to treat certain infections in specific hosts (Yusuf et al. 2023).

Fungi naturally produce antimicrobial compounds because, in their natural environments, their ability to inhibit the growth of other co-occurring microbes is essential to their survival (Chandra and Kumar 2017). Alkaloids, terpenoids, polyketides, polysaccharides, steroids, quinones, flavonoids, aliphatic compounds, and phenols are examples of metabolites having antibacterial activity that are produced by filamentous fungi belonging to the phylum Ascomycota (Blackwell 2011, Ramadan et al. 2023, Ramadan et al. 2024, Salem et al. 2022a,b).

When compared to laboratory-produced commodities, natural products have a wider range of chemical and biological structures. Antimicrobial and antibiofilm drugs can be developed by exploring these characteristics, which include antibacterial, anti-inflammatory, analgesic, antioxidant, and anticancer activities (Miller et al. 2022). Furthermore, natural compounds have been demonstrated to have fewer negative effects than laboratory-made products and to have antibacterial and chemopreventive properties in limiting the formation of biofilms (Lu et al. 2019).

Biofilms appear as a structure that helps in the ecological niche-establishing and survival strategies that bacteria have long since evolved. The microbial population is protected by biofilms, which also allow microbial aggregation, facilitate the spread of bacteria, and strengthen the bacteria's adaptability to external stresses (de Almeida Campos et al. 2023). Globally, biofilms are the source of high rates of morbidity and mortality, accounting for 65–80% of all human diseases, such as urinary tract infections, endocarditis, dental plaque, and cystic fibrosis (Vestby et al. 2020).

In the world, the third most common cause of cancer-related deaths globally is hepatocellular carcinoma (Sung and Jang 2018). Breast cancer is the most common malignant illness to affect women. It can spread to nearly every part of the body, most commonly the brain, liver, lymph nodes, and bones (Liu Cuiwei et al. 2022).

Numerous novel anticancer compounds have been identified and extracted from the secondary metabolic production of several fungi, including torreyanic acid from *Pestalotiopsis* sp. (Ding et al. 2009), vincristine from *Fusarium oxysporum* (Kumar and Ahmad 2013), *Aspergillus* sp.'s gliotoxin (Nguyen et al. 2013), Paclitaxel from *Penicillium aurantiogriseum* NRRL 62431 (Yang et al. 2014), and from *Aspergillus fumigatus* and *Alternaria tenuissima* (Ismaiel et al. 2017), camptothecin from *Fusarium solani* (Ran et al. 2017), and *Aspergillus terreus*'s Butenolides (Qi et al. 2018).

Consequently, the main objective of the current investigation was to assess the chemical composition of the *Aspergillus terreus* AUMC 15762 crude extract using GC-MS analysis, as well as antibacterial and antibiofilm activity against *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis.* In addition, antiproliferative activity of the crude extract was also evaluated on MCF-7 and HEPG2 cells.

Materials and Methods

Fungal strain isolation

The *Aspergillus* strain used in this study was isolated from a soil sample along the Sohag-Qena Road,

Sohag Governorate, Egypt, using the dilution plate method (Harris and Sommers 1968). The soil suspension was suitably diluted, followed by putting on Petri plates with Czapek's-Dox agar (CzA). The plates were then incubated at 25 °C for 10 days. After fungus development, the single spore isolation method (Choi et al. 1999) was used to purify the culture of CzA. After that, it was maintained in the culture collection of Assiut University Mycological Centre as AUMC 15762.

Morphological identification of the Aspergillus strain

Based on its macroscopic and microscopic characteristics, the *Aspergillus* strain AUMC 15762 used in this study was morphologically identified using several relevant sources (Moubasher 1993, Raper and Fennell 1965). The growth rates of the species were investigated on Czapek's agar (CZ; (Raper and Fennell 1965), malt extract agar (MEA; (Samson et al. 2010), and Czapek's yeast Autolysate agar (CYA; (Pitt 1979). Inoculations were made with spore suspensions suspended in a 0.2% agar and 0.05% Tween 80 solution (Samson et al. 2014). In a three-point design, plates were infected with an inoculum size of 1.0 µL/spot using a micropipette. After that, the plates were kept in the dark at 25 °C for seven days. The MEA culture's microscopic characteristics were examined.

Molecular identification of the Aspergillus strain

Fungal DNA was extracted using the method outlined by Moubasher et al. (2019), and the PCR reaction was conducted using SolGent EF-Taq (Al-Bedak and Moubasher 2020). The universal primers ITS1 and ITS4 were used to amplify the ITS region (White et al. 1990). DNASTAR (version 5.05) was used in this analysis to produce *Aspergillus* species sequences sequentially. GenBank provided the sequences of the most closely related *Aspergillus* species categorized in the Terri section. MAFFT (Katoh and Standley 2013) was used to align each sequence, and BMGE (Criscuolo and Gribaldo 2010) was used to optimize the alignment gaps and weak uninformative characters. Phylogenetic analyses using maximum-likelihood (ML) and maximum-parsimony (MP) methods were conducted using MEGA X (version 10.2.6) (Kumar et al. 2018). A 1000 replication test was used to determine how robust the most frugal trees were (Felsenstein 1985). Using Modeltest 3.7's Akaike Information Criterion (AIC), the optimal nucleotide substitution model for ML analysis was discovered (Posada and Crandall 1998).

Submerged fermentation conditions and crude extract preparation

Erlenmeyer conical flasks (250 mL) each containing 50 mL of sucrose-free Czapek's broth medium supplemented with 1.0 % tween 80 were used to culture *Aspergillus terreus* AUMC 15762. The medium contained (g/mL): sodium nitrate, 2; dipotassium hydrogen phosphate, 1.0; potassium chloride, 0.5; magnesium sulphate, 0.5; zinc sulphate, 0.01; copper sulphate, 0.005; and tween 80, 10. Each flask thereafter received one mL of spore suspension containing 1.5×10^8 spores/mL of the strain under investigation's 7-day-old culture. After fungal inoculation, the flasks were shaken at 150 rpm for seven days at 30 °C. Once the flasks had been incubated for 6 days, they were removed from the shaker and centrifuged at 10,000 rpm for 10 minutes at 4 ºC to get the cell-free supernatant. A freeze dryer (VirTis: Model 6 KBTES-55, NY, USA) was used to lyophilize the concentrated aqueous extract after it had been concentrated under reduced pressure.

GC-MS analysis of A. terreus AUMC 15762 crude extract

This investigation was carried out by the Analytical Chemistry Unit (ACAL), Faculty of Science, Assiut University, Egypt, to identify the active ingredients in *A. terreus* crude extract in in this study. Following the dissolution of 0.5 g of the *A. terreus* 15762 crude extract in 5.0 mL of methanol, the mixture was centrifuged at 10,000 rpm for 15 minutes at 5 °C. A Thermo-Scientific GC/MS (ISQ, 7890A-5975B, USA, MS: quadruple), was used to analyze 10 µL of the tested metabolites. The setup includes a 30 mm × 0.25 mm × 0.25 µm HP-5MS Capillary Standard nonpolar column. The cycle looked like this: With a post-run temperature of 260°C for 2 min, a flow program of 0.5 mL min for 10.9 min and then 1.0 mL/min for 30 min, an MS source of 230–250 °C, and an MS quad of 150–200 °C, the oven program was set to run for 48 min. The oven program started at 120 °C for 5.0 min, the maximum temperature was 280 °C, and the equilibration duration was set to 0.5.

Antibacterial properties of A. terreus AUMC 15762 crude extract

Crude extracts generated by *A. terreus* AUMC 15762 were evaluated for their antibacterial ability against four strains of pathogenic bacteria, namely *Escherichia coli* ATCC 8739, and *Serratia marcescens* AUMC B-89 (Gram-negative), *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228 (Gram-positive). To prepare the bacterial inoculum, strains were pre-cultured in nutrient broth for 24 hours at 37 °C under shacked condition of 150 rpm. Afterward, sterile cotton swabs were used to evenly distribute the bacterial suspensions (1.5×10^8 cells/mL = 0.5 McFarland standard

solution) on the surface of Petri plates that contained nutritional agar (Bhalodia and Shukla 2011). *A. terreus* AUMC 15762 crude extract was dissolved in distilled H_2O at concentrations of 20, 10, 5, and 2.5 mg/mL and tested in order to determine the minimum inhibitory concentrations (MIC). Using the well diffusion method (Valgas et al. 2007), five-mm-diameter wells were punched in the agar and individually added 50 µL of the crude extract under test. For 24 hours, the plates were incubated aerobically at 37±1 °C. After incubation, the bacterial growth suppression was determined in millimeters. There were three runs of the testing.

Effect of A. terreus AUMC 15762 crude extract on biofilm formation

In 96-well polystyrene flat bottom plates, the impact of *A. terreus* AUMC 15762' crude extract on biofilm formation was assessed (Antunes et al. 2010, Niu and Gilbert 2004). Essentially, 300 µL of wells were seeded with 10^6 CFU/mL of freshly inoculated Trypticase soy yeast broth (TSY). Sublethal quantities of minimal bactericidal concentration (MBC) (75, 50, and 25 %) were subsequently added to the wells and allowed to grow. As controls, wells with medium and wells with just methanol and no extracts were employed.

The plates were incubated at 37 °C for 48 hours. Following the incubation period, each well was thoroughly washed with sterile distilled water to make sure that no free-floating cells remained, and the supernatant was then collected. The biofilm that had developed on the plates was visible after 15 minutes of room temperature dyeing with a 0.1 % aqueous solution of crystal violet, after 30 minutes of air drying. To remove any remaining discoloration, the plate was rinsed with sterile distilled water three times after incubation. Then, each well was filled with 250 µL of 95 % ethanol to dissolve the dye adhered to the cells. After incubating for 15 minutes, absorbance was measured at 570 nm using a microplate reader (Mohanta et al. 2020) using the following Equation:

$$\% \text{ Biofilm inhibition } = \frac{1- (\text{Abs.of Sample} - \text{Abs.Blank})}{(\text{Abs.Control} - \text{Abs.Blank})} \times 100$$

Cytotoxicity of *A. terreus* AUMC 15762 crude extract on MCF–7 and HEPG2 cell lines

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

The 96-well tissue culture plate was seeded with 1 × 10^5 cells/mL (100 µL/well) and then incubated at 37 °C for 24 hours to generate a cell monolayer. A 20 µL of the MTT solution was added to each well, and the plate was incubated for 24 hours at 37 ºC and 5.0 % CO_2 conditions (Bio Basic Canada. Inc) (Mosmann 1983, Slater et al. 1963). Once a confluent cell layer developed, the growth material was removed, and the media was washed twice to remove the cell monolayer. The control group consisted of three wells that received only RPMI medium. A distinct well was used to test the 0.1 mL of each concentration from the *A. terreus* AUMC 15762 crude extract. After the plate was incubated at 37 °C, it was examined. The optical density was determined at 560 nm (Alley et al. 1988, Van de Loosdrecht et al. 1994).

Results

Morphological and molecular identification of the Aspergillus AUMC 15762 strain

The *Aspergillus* strain utilized in this investigation had the same physical traits as *A. terreus*, which produces colonies that ranged in color from cinnamon to orange-brown and had long, compact, columnar conidial heads carried on short, biseriate conidiophores (Fig 1).

A phylogenetic study based on ITS sequencing was employed for molecular confirmation of the *Aspergillus terreus* strain in this study. In the final ITS data set, 15 sequences produced 605 characters altogether, of which 512 could be successfully aligned, 57 were categorized as variable, and 16 were categorized as informative. The best model to illustrate the relationship between taxa was Tamura's 3-parameter model (T92). The Maximum Parsimony method produced 10 trees. Tree length of 70, greatest log likelihood of -1221.77, consistency index of 0.916667, retention index of 0.954545, and composite index of 0.875000 are the features of the most parsimonious tree (Fig 2). The strain under investigation is situated at the same branch as *A. terreus* NRRL 255 (Fig 2).

Fig 1. *Aspergillus terreus* AUMC 15762. (A) Seven-day-old colonies on CYA at 25 °C (B) Conidiophores and columnar conidial heads (C) Smooth, globose conidia (Scale bars = 20 µm).

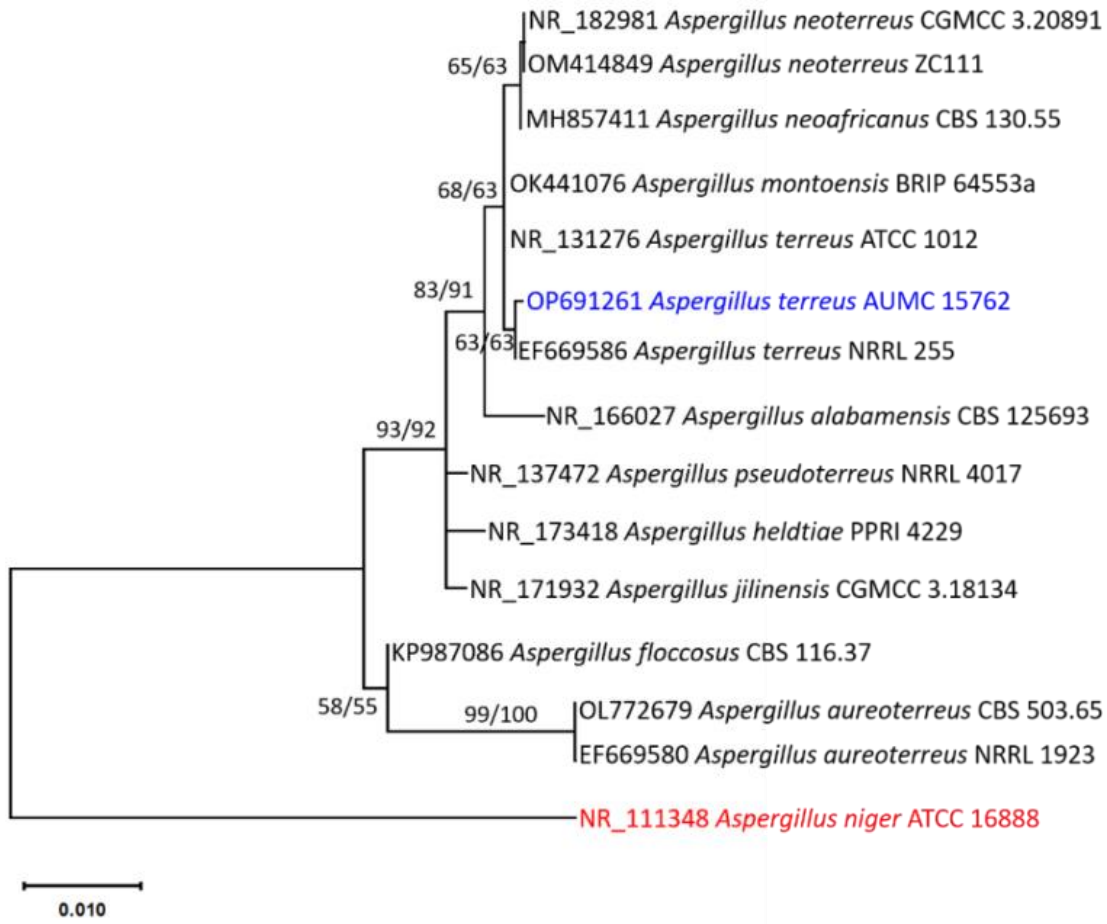

Fig 2. Maximum likelihood phylogenetic tree produced by ML/MP analysis of *Aspergillus terreus* AUMC 15762 ITS sequences in this work (shown in blue) in comparison to the *Aspergillus terreus* group's most closely related species in GenBank. Near the corresponding nodes are the bootstraps (1000 replications) with ML/MP ≥ 50%. *Aspergillus niger* ATCC 16888 is the source of the tree's roots (in red).

GC-MS analysis of A. terreus AUMC 15762 crude extract

The composition of *A. terreus* AUMC 15762 crude extract was identified using GC-MS qualitative analysis. Based on the fragmentation pattern, molecular weight, and retention time, the current results showed the presence of 25 compounds (Fig 3; Table S1). The most prevalent compounds were ten fatty acids (Elaidic acid, 2-Amino-5-Guanidino-Pentanoic Acid, Succinic acid, lauric acid, Thapsic acid, Methyl 7,10-hexadecadienoate, n-Decanoic acid, Nonanoic acid, Octadecanedioic acid, and Lignoceric acid) comprising 40 % of total. Three Polyethylene Oxides (Octaethylene glycol, Pentaethylene glycol and 1,4,7,10,13,16-Hexaoxacyclooctadecane) constituting 12.0 % of total. Three Monosaccharide derivatives (1,6-Anhydro-.beta.-D-glucofuranose, D-Allose and Sorbitol) constitute 12.0 % of the total. Two Tertiary amines (N,N-Dimethylformamide diisopropyl acetal and N,N-Dimethylthiourea) account for 8.0 % of the total. Alkaloids (4-Amino-1h-Imidazole-5-Carboxamide), Ethylbenzenes ((2,2-diethoxyethyl)-Benzene), Phenolic derivatives (2-[2-(4-Nitrophenoxy) ethoxy]ethanol), Furan derivatives (Hydroxymethylfurfural), Pyranones derivatives (3-Hydroxy-2,6-Dimethyl- 4h-Pyran-4-One), Oxygenated Hydrocarbons (Grandisol) and Halogenated alcohol (Glycol bromohydrin), were also detected comprising 4.0 % each. It was shown that the identified compounds had antibacterial, cytotoxic, antioxidant, and anti-inflammatory properties (Table S1).

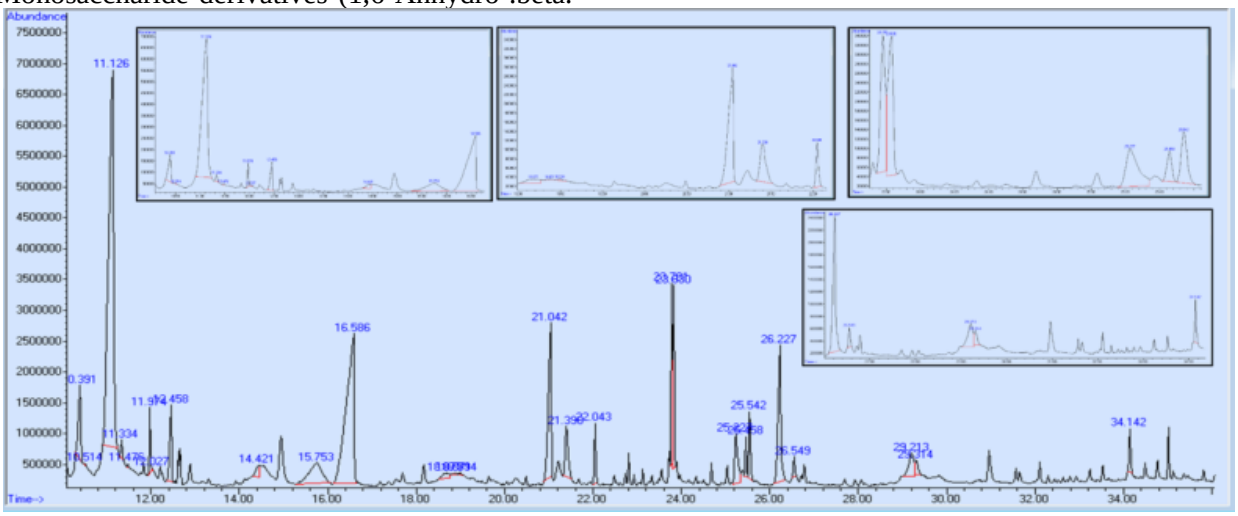

Fig 3. GC-MS chromatogram of *A. terreus* AUMC 15762 crude extract.

Antibacterial properties of A. terreus AUMC 15762 crude extract

The current findings demonstrated that all concentrations used (20, 10, 5, and 2.5 mg/mL) from the *A. terreus* AUMC 15762 crude extract efficiently suppressed bacterial growth. The effect was gradually decreased by decreasing the concentration. The lowest concentration (2.5 mg/mL) displayed 20±1, 18±2, 19±2, and 20±1 mm inhibition on *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis*, respectively (Table 1; Fig 4).

Table 1 The antibacterial properties of the *A. terreus* AUMC 15762 crude extract against Gram-positive and Gram-negative bacterial strains (mean±SD, *n* = 3).

	A. terreus AUMC 15762 crude extract				Chloramphenicol			
Bacterial strains	20.0 mg/mL (mm)	10.0 mg/mL (mm)	5.0 mg/mL (mm)	2.5 mg/mL (mm)	20.0 mg/mL (mm)	10.0 mg/mL (mm)	5.0 mg/mL (mm)	2.5 mg/mL (mm)
E. coli	30±2	27±1	25±2	20±1	25±3	15±3	9±1	0
S. marcescens	30±3	25±2	22±1	18±2	25±4	20±2	13±3	0
S. aureus	35±3	30±2	25±3	19±2	15±2	10±3	0	0
S. epidermidis	30±4	27±3	25±3	20±1	26±3	20±2	17±1	15±4

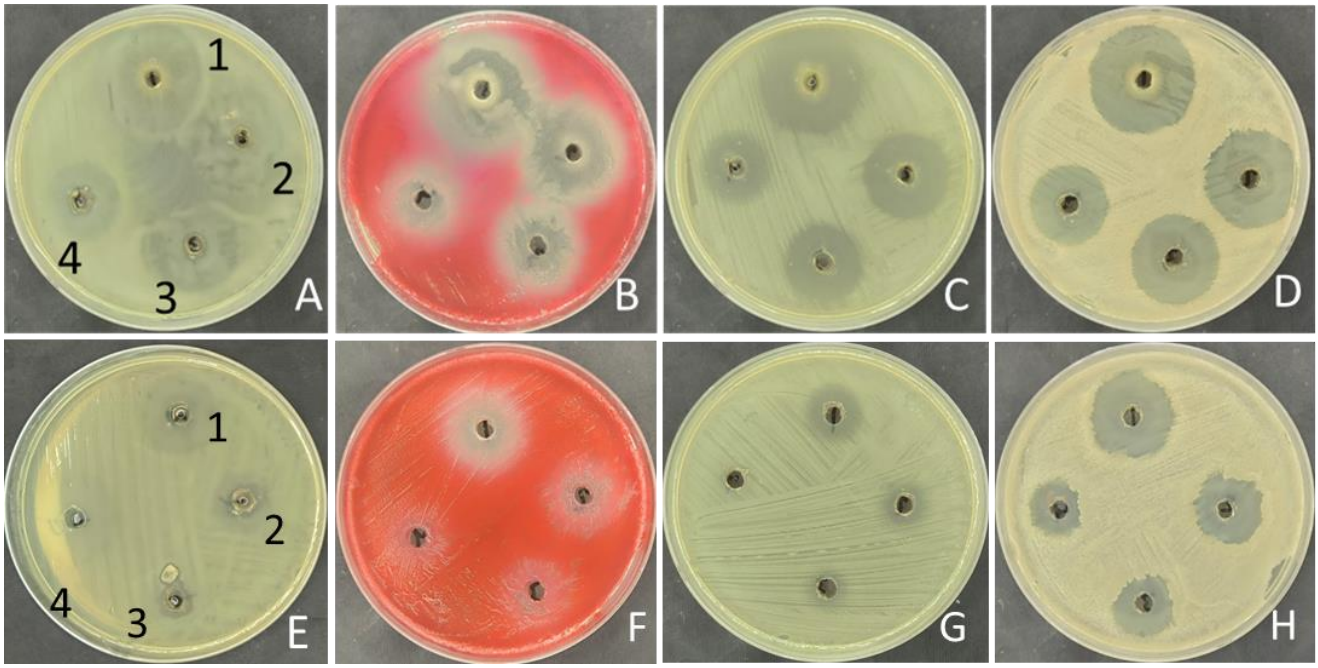

Fig 4. Antibacterial effect of (A–D) *A. terreus* AUMC 15762 crude extract against *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis* (1 = 20 mg/mL, 2 = 10 mg/mL, 3 = 5.0 mg/mL, 4 = 2.5 mg/mL). (E–H) Chloramphenicol on the tested bacteria *E. coli*, *S. marcescens*, *S. aureus* and *S. epidermidis*, respectively.

Effect of A. terreus AUMC 15762 crude extract on biofilm formation

Findings of the present study demonstrated the effectiveness of *A. terreus* AUMC 15762 crude extract at 75, 50, and 25 % of MBC in reducing the amount of biofilm that was formed by *E. coli* (92.69 %, 90.23 %, and 84.69 %), *S. marcescens* (92.0 %, 91.1 %, and 84.6 %), *S. aureus* (89.17 %, 85.4 %, and 76.95 %), and *S. epidermidis* (93.46 %, 88.56 %, and 80.71 %), respectively (Figure 5; Table S2).

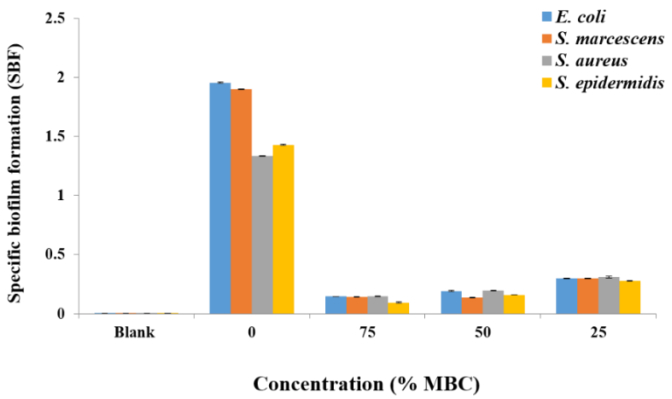

Fig 5. Effect of *A. terreus* AUMC 15762 crude extract on biofilm formation by *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis*.

Cytotoxicity of A. terreus AUMC 15762 crude extract

The MCF-7 and HEPG2 cell lines (Fig 6) were used to determine the anticancer properties of the *A. terreus* AUMC 15762 crude extract. On MCF-7 cells, the cytotoxicity significantly increased with increasing the concentrations of *A. terreus* AUMC 15762 crude extract (Fig 7). The increases ranged from 0.086–97.7 %, Treatment of the HEPG2 cell lines by *A. terreus* AUMC 15762' crude extract significantly induced cytotoxicity ranging from 0.0–97.55 % (Fig 8). IC_{50} exhibited 156.84±2.15 µg/mL and 120.59±1.96 µg/mL for MCF-7 and HEPG2 cell lines, respectively (Fig 9).

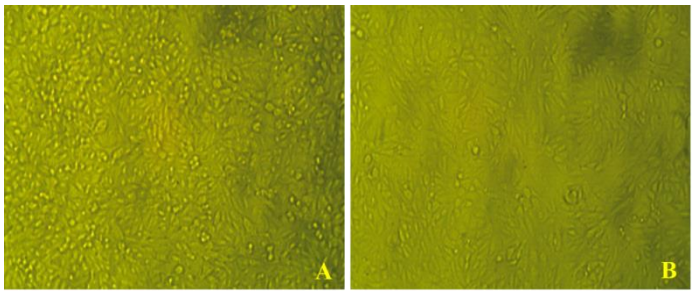

Fig 6. (A) MCF-7 and (B) HepG2 cell lines before treatment with *A. terreus* AUMC 15762 crude extract

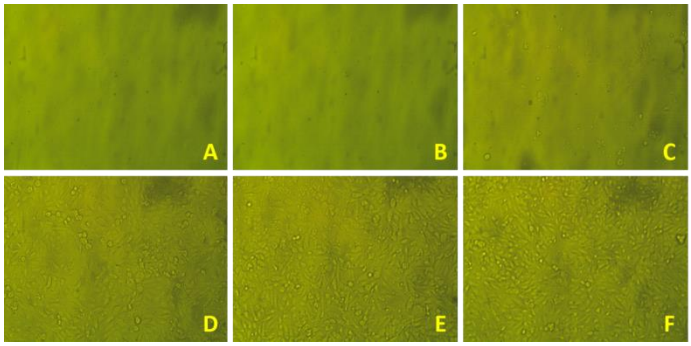

Fig 7. Effect of different concentrations (A) 1000 µg/mL, (B) 500 µg/mL, (C) 250 µg/mL, (D) 125 µg/mL, (E) 62.5 µg/mL, and (F) 31.25 µg/mL of *A. terreus* AUMC 15762 crude extract on MCF-7.

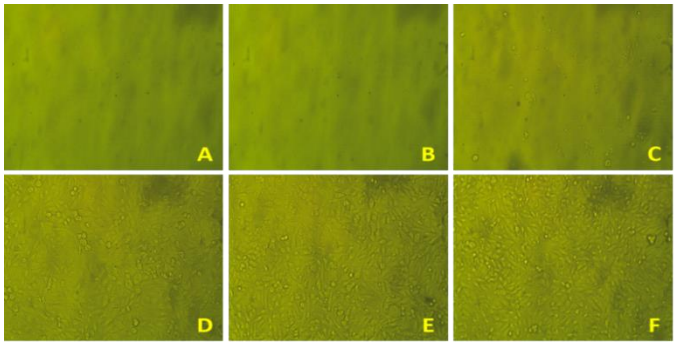

Fig 8. Effect of different concentrations (A) 1000 µg/mL, (B) 500 µg/mL, (C) 250 µg/mL, (D) 125 µg/mL, (E) 62.5 µg/mL, and (F) 31.25 µg/mL of *A. terreus* AUMC 15762 crude extract on HEPG2 cell line.

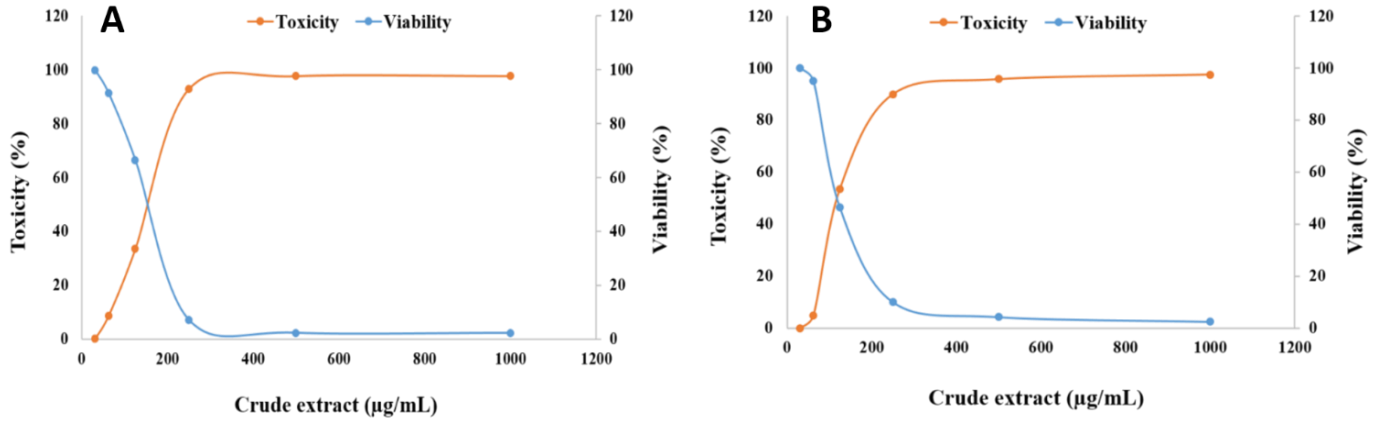

Fig 9. Toxicity effect of *A. terreus* AUMC 15762 crude extract on viability of (A) MCF-7 cell line. (B) HEPG2 cell line.

Discussion

The composition of the crude extract of *A. terreus* AUMC 15762 was ascertained by GC-MS analysis. Based on the retention time, molecular weight, and fragmentation pattern, the current data showed the existence of 25 compounds. In this regard, it has been demonstrated that several fungi create a wide range of secondary metabolites, including tertiary amines, amino acids, fatty acids, aromatic compounds, and alkaloids (Ancheeva et al. 2020, Chen et al. 2022, Demain 2014, Devi et al. 2020, Kamat et al. 2023, Pinar and Rodríguez-Couto 2024, Rateb and Ebel 2011, Singh and Kumar 2023). The secondary metabolites that were studied showed that alkaloids had a strong antibacterial effect (Othman, Sleiman, & Abdel-Massih, 2019). According to Casalvas-Vargas et al. (2021), one naturally occurring FA that possesses considerable antibacterial properties is lauric acid.

Regrettably, an increasing number of pathogenic bacteria have lately evolved resistance to conventional antibiotics as a result of prolonged usage and/or overuse. The search for substitutes for traditional antibiotics is desperately needed everywhere. As demonstrated in this manuscript, the crude extract derived from *A. terreus* AUMC 15762 exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Specifically, the extract significantly inhibited the growth of *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis* in the current investigation.

Regarding this, it was demonstrated that *Trichoderma harzianum* CBMAI 43, *Guignardia* sp. CBMAI 69, and *Phomopsis* sp. CBMAI 164 suppressed the growth of the human pathogenic bacteria *E. coli*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, and *S. aureus* (Sette et al. 2006). Numerous biologically active compounds have been identified in *Aspergillus terreus*, including alkaloids (El-Hawary et al. 2021, Qi et al. 2020), butyrolactone I (Ghfar et al. 2021, Hamed et al. 2020), terpenoids (Girich et al. 2020, Uras et al. 2021) and polyketides (Deng et al. 2020, El-Hawary et al. 2021).

These substances provide a rationale for the *Aspergillus* genus considerable significance in the scientific and pharmaceutical industries levels (Zhang et al. 2018). Additionally, it has been proven that a number of secondary metabolites produced by fungi has antibacterial characteristics (Al-Jassaci et al. 2016, Hateet et al. 2014, Li et al. 2014, Liu Xiao-Bo et al. 2019).

In this investigation, the *A. terreus* AUMC 15762 crude extract exhibited significant antibiofilm activity against both Gram-positive and Gram-negative bacteria. Ordinary bacterial populations encased in an extracellular polymeric material (EPS) matrix containing various exopolysaccharides, lipids, secreted proteins (some of which can form amyloid fibrils), and extracellular DNA are known as bacterial biofilms (Flemming and Wuertz 2019). Bacterial biofilms are necessary to keep dangerous bacteria from being destroyed by conventional antibiotics (Sharma et al. 2019).

Because of the bacterial cell aggregation caused by bacterial biofilms, the wound-healing process is significantly slowed down (Mancl et al. 2013). This style of bacterial development raises the level of resistance to standard antibiotics and is linked to 65–80 % of all clinical infections (Pletzer and Hancock 2016). Antimicrobial drugs cannot penetrate the biofilm most of the time because of the presence of extracellular polymeric substance (EPS), which acts as a barrier to protect the bacterial cells within the biofilm. The use of chemicals that can degrade biofilm EPS will thus be the remedy (Kalpana et al. 2012). Antibacterial drugs that block biofilm resistance may be more effective in eliminating biofilm-related infections that are resistant to current treatments (Stewart 2002).

Scientists have employed a range of bio-active and chemically created compounds to prevent the growth of biofilms harboring pathogenic bacteria (Khan et al. 2018, Khan et al. 2019, Khan et al. 2020c, Mulat et al. 2019). Additionally, reviving aminoglycosides has been used to prevent biofilm development and harmful bacterial infections (Khan et al. 2020a). Given that attachment was an essential step in the formation of biofilms, attenuating motility properties may be a very promising method of process management (Khan et al. 2020b, Khan et al. 2020d). Using enzymes is a wonderful technique to remove biofilms because they are very ecologically friendly and degradable (Elamary and Salem 2020, Xavier et al. 2005).

In this study, at varying dosages of *A. terreus* AUMC 15762 crude extract, the SBF of *E. coli*, *S. marcescens*, *S. aurous*, and *S. epidermidis* fell to < 1.0 from 1.957, 1.9, 1.338, and 1.433, respectively. Based on specific biofilm formation (SBF), Mittal et al. (2010) classified biofilm producers into three categories: weak biofilm producers (SBF index < 1.00), intermediate biofilm producers (SBF index < 2.00), and robust biofilm producers (SBF index > 2.00). Drugs that alter calcium binding, in particular, may also affect the production of biofilms since divalent cations, especially calcium, are essential bridge ions for the creation of bacterial polysaccharides and biofilms and have regulatory functions in bacterial gene expression (Das et al. 2014).

Cytotoxicity, which can provide information on cell viability, death, and metabolic activities, is one of the most important indicators for biological assessment *in vitro* study (Aslantürk 2018). In this study, cytotoxicity of the crude extract generated by *A. terreus* AUMC 15762 was examined using the HEPG2 and MCF-7 tumor cell lines. Having IC_{50} values of 120.59±1.96 and 156.84±2.15 µg/mL, respectively. The cytotoxic potential of crude extracts derived from several fungi has been assessed in cell lines of various kinds. This is why *Aspergillus* sp. TRL1 produces the anticancer compound pulchranin, which efficiently inhibits human tumor cells like the liver (Hep-G2) and breast (MCF-7) cell lines (Moussa et al. 2020). 9-octadecenoic acid is an omega-9 unsaturated fatty acid that has several positive effects on human health (Ghavam and Manca, 2021). The fact that unsaturated fatty acids activate cholesterol acetyltransferase to reduce cholesterol is well-known. Fats help with hypertension, cancer, heart disease, autoimmune diseases, Parkinson's disease, Alzheimer's disease, inflammation, and Parkinson's. Cheque and Legrand (2014), Ghavam et al. (2021), and Sales-Campos, Crema Peghini, Santana da Silva, and Ribeiro Cardoso (2013) are among the studies that have used its compounds as an anticancer treatment due to their potential to alter the cell membrane and cause cancer cells to undergo apoptosis. The crude extract of a sterile algicolous fungus (KT31), isolated from the red seaweed *Kappaphycus alvarezii* exhibited potent cytotoxic activity with an IC50 value of 1.5 µg/mL (Tarman et al. 2011). The crude extracts derived from marine fungi have shown cytotoxic effects against human lung carcinoma (A-549), human cervical carcinoma (HeLa), and human hepatoma (HepG2) cell lines (Zhao et al. 2018). Strong cytotoxic activity against the murine leukemic P388 cell line and 1.7% against the human chronic myeloid leukemic cell line K562 was demonstrated by 3.3 % of 300 endophytic fungi' crude extracts. Strong cytotoxicity was displayed by *Sporothrix* sp. against human breast adenocarcinoma (MCF7) and colorectal cancer (HCT116) cell lines (Hazalin et al. 2009). The crude extract of *Alternaria* sp. KTDL7 exhibited notable cytotoxicity on UMG87 glioblastoma cells (Tapfuma et al. 2019).

Conclusions

In conclusion, GC-MS analysis was utilized in order to evaluate the chemical composition of the aqueous extract that was produced by *A. terreus* AUMC 15762 in a liquid media. Twenty-five compounds were identified through the use of GC-MS analysis. These compounds included twelve compounds related to fatty acids, three monosaccharide derivatives, two tertiary amines, alkaloid, ethylbenzenes, polyethylene oxides, phenolic derivatives, furan derivatives, pyranone derivatives, oxygenated hydrocarbons, and halogenated alcohol (one compound each). The aqueous extract of *A. terreus* exhibited potential antibacterial and antibiofilm capabilities against strains of *E. coli*, *S. marcescens*, *S. aureus*, and *S. epidermidis*. In addition, the aqueous extract of *A. terreus* has been shown to elicit a significant cytotoxic effect against the MCF-7 and HEPG2 cell lines.

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