Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.7.2.4

Comparative Assessment of Antibacterial, Antifungal and Cytotoxic Potential of *Aspergillus fumigatus* and *Penicillium chrysogenum* Extracts

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Abstract

Crude extracts derived from *Aspergillus fumigatus* and *Penicillium chrysogenum*, isolated from soil samples, were investigated for their antibacterial, antifungal, and cytotoxic activities. Ethyl acetate (EtoAc) and n-hexane (n-Hex) fractions of the extracts were tested against six pathogenic bacterial strains, including three gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*) and three gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*), as well as six fungal pathogens comprising three yeasts (*Candida albicans*, *C. tropicalis*, *Rhodotorula*) and three mycelial fungi (*Trichophyton rubrum*, *Fusarium oxysporum*, *Microsporum canis*). Cytotoxic activity was evaluated using brine shrimp lethality assay. The EtoAc fractions of both fungi demonstrated significantly stronger antimicrobial activity than their n-Hex fractions. The EtoAc fraction of *A. fumigatus* exhibited pronounced antibacterial effects, with *S. aureus* showing the highest susceptibility (58 mm inhibition zone at 500 µg/mL), followed by *B. subtilis* (48 mm) and *S. pyogenes* (45 mm). Gram-negative bacteria displayed moderate susceptibility, with *S. enterica* (35 mm) and *E. coli* (30 mm) being inhibited, while *P. aeruginosa* exhibited minimal response (20 mm). The n-Hex fraction showed reduced efficacy, with *S. enterica* (40 mm) being the most susceptible. For antifungal activity, the EtoAc fraction of *A. fumigatus* demonstrated robust inhibition, particularly against *M. canis* (60.5%) and *T. rubrum* (54%), while *Rhodotorula* was the most susceptible yeast (52%). The EtoAc fraction of *P. chrysogenum* displayed exceptional antifungal potential, with maximum inhibition against *T. rubrum* (70%) and *M. canis* (63.5%). The n-Hex fractions of both fungi exhibited limited antifungal activity at higher concentrations. Cytotoxicity assays revealed dose-dependent effects, with the EtoAc fraction of *A. fumigatus* inducing mortality rates of 3.33%, 46.66%, and 71.6% at 10, 100, and 1000 µg/mL, respectively. The n-Hex fraction exhibited maximum lethality of 53.33% at 1000 µg/mL. Similarly, the EtoAc fraction of *P. chrysogenum* displayed moderate cytotoxicity (46.66% at 1000 µg/mL), while its n-Hex fraction exhibited the highest lethality (83.33%).

Keywords: *Artemia salina*, Bioassay screening, *Candida*, *Microsporum*, Secondary metabolites.

Article Info:
Received:
December 10, 2024
Received Revised:
December 27, 2024
Accepted:
January 03, 2025
Available online:
January 08, 2025

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1. INTRODUCTION

The growing threat of drug-resistant pathogens, responsible for severe and often life-threatening infections, has significantly increased the urgency to discover novel antibiotics. The development of such medications is pivotal in combating infectious diseases, yet the rising prevalence of antimicrobial resistance continues to challenge modern medicine [1]. Although bioactive compounds can be synthesized chemically, natural sources particularly microorganisms remain unparalleled in their ability to produce structurally diverse and therapeutically potent secondary metabolites [2]. The revolutionary discovery of penicillin in 1928, derived from *Penicillium chrysogenum*, marked a transformative moment in medical history, highlighting fungi as invaluable reservoirs of antimicrobial agents [3,4]. Since that breakthrough, fungi have remained central to natural product research, yielding numerous bioactive compounds with therapeutic significance over recent decades [5].

Fungi, particularly those isolated from soil environments, are recognized as prolific producers of secondary metabolites, a reflection of their ecological adaptability and metabolic versatility. Soil fungi, thriving in highly competitive and nutrient-variable ecosystems, produce secondary metabolites that serve as chemical defences or signalling molecules, often with potent biological activity [6,7]. Beyond soil fungi, plant-associated fungi exhibit exceptional biological diversity and synthesize chemically distinct and biologically active metabolites as a result of their intricate interactions with host plants [8,9]. These dual niches i.e. soil and plant-associated ecosystems, offer immense potential for the discovery of novel bioactive substances with therapeutic applications.

Among fungi, the genus *Aspergillus* is especially noteworthy, encompassing approximately 180 species known for their remarkable metabolic capabilities [10, 11]. Members of this genus produce a wide array of biologically active compounds with diverse pharmacological properties, including anticancer and immunosuppressive agents [12]. Additionally, certain *Aspergillus* species, such as *A. fumigatus*, are recognized for producing secondary metabolites like gliotoxin and fumagillin, which exhibit potent antimicrobial, antifungal and cytotoxic activities [13]. The genus also demonstrates versatility in synthesizing plant growth-promoting hormones such as gibberellins [14]. Similarly, the genus *Penicillium*, comprising about 200 species primarily inhabiting terrestrial ecosystems, has made substantial contributions to natural product discovery [15, 16]. Beyond penicillin, *Penicillium* species have yielded a range of bioactive compounds, including griseofulvin, patulin, and other metabolites, many of which remain valuable drug leads [17].

This study focuses on *Aspergillus fumigatus* and *Penicillium chrysogenum*, two fungal species isolated from agricultural topsoil collected from various locations. These fungi were selected based on their potential to produce bioactive metabolites under controlled laboratory fermentation conditions. The primary objective was to evaluate the biological activity of crude extracts derived from these fungi through bioassay-guided screenings. Specifically, the ethyl acetate and n-hexane fractions of the crude extracts were subjected to antibacterial, antifungal and cytotoxic assays. The aim was to identify metabolites with therapeutic

potential, offering insights into their possible applications in combating infectious diseases and other health challenges.

2. MATERIALS AND METHODS

2.1 Isolation and purification of the fungal strains

To obtain fungal isolates, soil samples were collected from different locations of the agricultural farm of the University of Agriculture Peshawar. The soil dilution plate method, as described by Takahashi et al. (2008), was employed for the isolation process. Diluted soil samples were inoculated onto Potato Dextrose Agar (PDA) plates and incubated at 28°C for ten days [18]. Following incubation, emerging fungal colonies were sub-cultured to achieve pure strains for further study.

2.2 Microscopic and morphological identification of the isolated fungal strains

The morphological characteristics of the purified fungal isolates were analysed using a light microscope at 40-1000x magnification. Identification was based on key morphological features, including hyphal structure, colony architecture, and spore arrangement. The coloration of fungal colonies was documented by culturing the isolates on Potato Dextrose Agar (PDA) and Czapec Dox Agar (CDA) media. These observations provided preliminary insights into the identity of the fungal strains [19, 20]. For further validation, the fungal isolates were submitted to the Department of Plant Pathology at the University of Agriculture Peshawar, where expert analysis was conducted to confirm their identification.

2.3 Culturing of fungi in modified Czapec broth medium for production of metabolites

The fungal isolates were cultured on Czapec Dox Agar (CDA) medium, prepared with 1% peptone, 1% glucose, 0.05% magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.05% potassium chloride (KCl), and 0.001% ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), with the pH adjusted to 7.3 ± 0.2. To enhance metabolite production, the medium was supplemented with 3% starch and 2% additional glucose. The fungal strains were then inoculated into the prepared medium and incubated in a shaking incubator at 28°C with continuous shaking at 150 rpm for 14 days, ensuring optimal conditions for metabolite synthesis [21, 22].

2.4 Extraction and concentration of fungal metabolites

To facilitate separation of media components and promote sedimentation, approximately 200–250 µL of 40% concentrated HCl was added to each flask. The fungal mycelia were then homogenized using an electric blender, followed by the addition of an equal volume of ethyl acetate to each flask. The mixture was intermittently shaken for 30 minutes to ensure thorough mixing. The homogenized mycelia were filtered using cheesecloth to separate the solid components. The resulting mixture was transferred to a separating funnel to recover the organic layer, which was subsequently washed with 2M brine solution to remove impurities. The organic phase was dehydrated by adding anhydrous sodium sulfate (Na_2SO_4) to eliminate residual water.The dehydrated organic layer, containing crude metabolites, was filtered again

and concentrated using a rotary evaporator at 45°C. The dried crude extract was then collected from the rotary evaporator flask by rinsing with methanol, yielding the final metabolite preparation [23].

2.5 Bioassay screening of the fungal crude metabolites

The bioactive potential of the fungal crude metabolites was evaluated through antibacterial, antifungal and cytotoxic bioassays. For this purpose, the ethyl acetate and n-hexane fractions of the crude extracts from both fungal species were analysed.

2.5.1 Antibacterial activity

The antibacterial activity of crude ethyl acetate and n-hexane fractions from *Aspergillus* and *Penicillium* species was tested against six pathogenic bacterial strains, including three gram-positive (*Staphylococcus aureus, Bacillus subtilis* and *Streptococcus pyogenes*) and three gram-negative species (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica*). The agar well diffusion method was used to evaluate the antibacterial potential. Fresh cultures of the bacteria were prepared by inoculating each strain into 1 mL of sterile nutrient broth (NB), which had been autoclaved at 121°C. The cultures were incubated at 37°C for 24 hours. Nutrient agar (NA) medium was then prepared, and 22 mL was poured into sterile Petri plates. Once solidified, wells were created in the agar using a sterile metal borer with appropriate spacing. Bacterial suspensions were adjusted to 0.5 McFarland's turbidity standard to ensure uniformity. A volume of 20 µL of each bacterial culture was evenly spread on the nutrient agar plates to create a uniform lawn. Stock solutions of the fungal extracts were prepared by dissolving them in dimethyl sulfoxide (DMSO) at a concentration of 12 mg/mL. From these stock solutions, various concentrations (10, 100, 200, 250, and 500 µg/mL) were prepared, and equal volumes were added to the corresponding wells. Ciprofloxacin, at a concentration of 100 µg/mL, was selected as the positive control due to its broad-spectrum activity against both gram-positive and gram-negative bacteria. Its well-documented efficacy and standardized breakpoints, as outlined in the CLSI guidelines, ensure accurate and reliable benchmarking for interpreting the antibacterial potential of the fungal extracts. The plates were left at 25°C for 2–3 hours to allow proper diffusion of the samples into the agar, followed by incubation at 37°C for 24 hours [24].

2.5.2 Antifungal activity

The antifungal activity of the fungal crude extracts was assessed using the agar tube dilution method against six fungal pathogens comprising three yeast species (*Candida albicans, Candida tropicalis* and *Rhodotorula*) and three mycelial fungi (*Trichophyton rubrum, Fusarium oxysporum* and *Microsporum canis*). A stock solution of 24 mg/mL was prepared in sterile dimethyl sulfoxide (DMSO). Potato Dextrose Agar (PDA) medium was prepared following the manufacturer's instructions using sterile distilled water, and 5 mL of the medium was dispensed into screw-capped test tubes. The tubes were autoclaved at 121°C for 15 minutes and then cooled to 35°C. In the non-solidified medium, test samples were added at concentrations of 10, 100, 250, 500, and 1000 µg/mL, prepared from the stock solution. The medium was then allowed to solidify in a slanted position at room temperature. After solidification, each tube was inoculated with a small fungal plug (4 mm in diameter) taken from a seven-day-old culture. Miconazole, at

a dose concentration of 100 µg/mL, was used as the standard antifungal drug for comparison. Miconazole was used as the standard antifungal drug for comparison. It was chosen due to its broad-spectrum activity against both yeast and filamentous fungi and its established role in antifungal susceptibility testing. Its mode of action, targeting ergosterol biosynthesis, provides a robust benchmark for evaluating the antifungal potential of the fungal crude extracts. The tubes were incubated at 28°C for seven days, during which fungal growth inhibition was visually assessed to evaluate the antifungal efficacy of the test samples [25, 26].

2.5.3 Brine shrimp cytotoxicity assay

To assess cytotoxicity, *Artemia salina* eggs were hatched in an artificial sea medium prepared by dissolving 3.8 g of sea salt in 1 liter of double-distilled water. Approximately 10 mg of eggs were added to the medium, and the setup was maintained under ordinary light at 25°C. After two days, the hatched larvae (nauplii) were collected for the assay. A stock solution of the test samples was prepared using ethanol as the solvent at a concentration of 20 mg/mL. From this stock solution, dose concentrations of 10, 100, and 1000 µg/mL were prepared. Each concentration was tested in a separate tray containing 15 brine shrimp larvae and artificial sea medium. After 24 hours, the number of surviving shrimp in each tray was recorded to evaluate the lethality of the test samples. The same experiment was repeated with potassium dichromate which was used as control [27]. The experiment was conducted in triplicate to ensure reliability and reproducibility of the results.

3. RESULTS

3.1 Description of the morphological and microscopic features of the fungal isolates

The colonies of *Penicillium* exhibited a velvety or woolly texture with a characteristic coloration that combined white and dark grey tones when cultured on Potato Dextrose Agar (PDA). The surface of the colonies had a powdery appearance, likely due to the presence of a dense spore layer. Microscopic examination of the fungal slides revealed septate hyphae forming the mycelial structure. The hyphae appeared translucent, with some displaying a faint greenish or bluish hue. At the tips of the hyphal branches, clusters of conidia were observed. These conidia were predominantly oval to cylindrical in shape and, in some cases, were linked in chains by delicate sterigmata. The colonies of *Aspergillus* initially appeared white or pale yellow on PDA, gradually changing to yellowish-green or olive-green as they matured. The colony surface was smooth with a velvety texture. Microscopic analysis revealed the distinctive fruiting bodies of *Aspergillus*. The conidiophores were elongated, unbranched, and cylindrical, bearing clusters of oval-shaped conidia that were arranged in chains. Together, the conidiophores and conidia formed well-defined conidial heads, a hallmark feature of the genus *Aspergillus*.

3.2 Result of bioassay screening

3.2.1 Antibacterial activity

The antibacterial activity of the ethyl acetate fraction of *Aspergillus* was evaluated against six pathogenic bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa*) at various concentrations ranging from 10 to 500 µg/mL. Among the strains, *S. aureus* exhibited the highest susceptibility, with inhibition zone reaching approximately 58 mm at the highest tested concentration (500 µg/mL). The inhibition gradually increased with increasing concentrations, indicating strong antibacterial potential against this gram-positive pathogen. Similarly, *B. subtilis* and *S. pyogenes* showed significant inhibition, with maximum inhibition levels of around 48 mm and 45 mm, respectively, at 500 µg/mL. These results suggest that the ethyl acetate fraction is particularly effective against gram-positive bacteria.

In contrast, gram-negative strains such as *S. enterica* and *E. coli* displayed moderate susceptibility. The maximum inhibition zone observed for *S. enterica* was approximately 35 mm, while *E. coli* showed about 30 mm zone of inhibition at the highest concentration. These findings indicate that while the fraction is less effective against gram-negative bacteria, it still demonstrates measurable antibacterial activity. *P. aeruginosa*, however, was the least affected, with minimal inhibition observed across all tested concentrations. The maximum inhibition zone recorded for this strain was 20 mm at 500 µg/mL, suggesting that it is highly resistant to the bioactive components present within the ethyl acetate fraction. Overall, the ethyl acetate fraction of *A. fumigatus* displayed broad-spectrum antibacterial activity, with stronger effects against gram-positive bacteria compared to gram-negative bacterial strains (Figure 1).

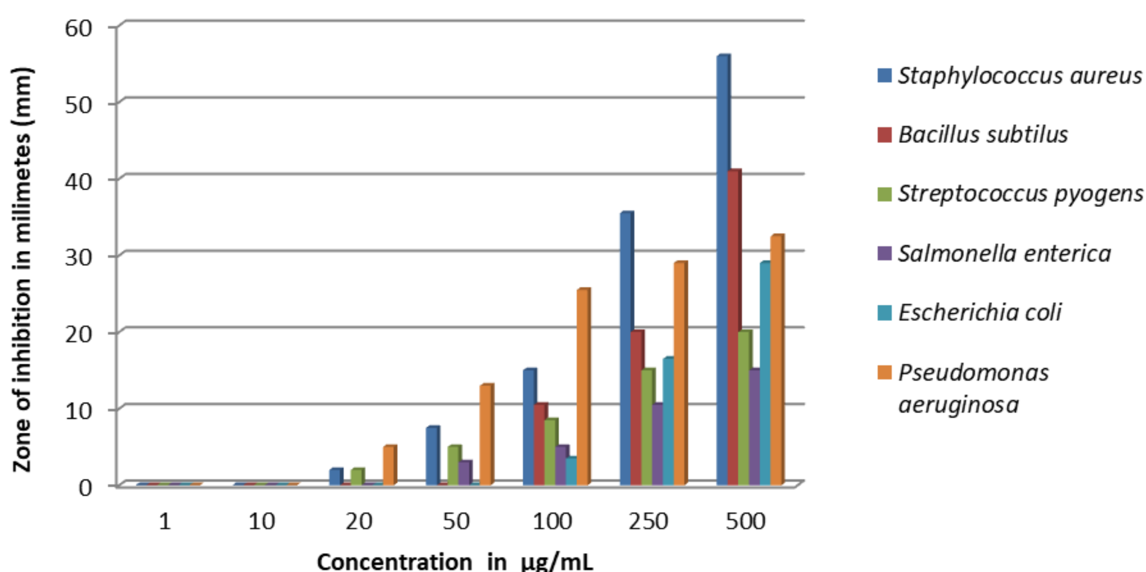

Fig. 1. Antibacterial activitity of ethyl acetate fraction of *A. fumigatus*.

Similar to the ethyl acetate fraction, the n-hexane fraction exhibited a concentration-dependent antibacterial effect, though its activity was generally lower. Among the tested strains, *S. aureus* demonstrated moderate susceptibility, with maximum inhibition zone reaching 10.5 mm at 500 µg/mL. *B. subtilis* exhibited comparable inhibition, peaking at 10 mm at the same concentration. *S. pyogenes*,

however, was less affected, with inhibition zones reaching only 5 mm at 500 µg/mL, indicating weaker activity against this gram positive bacterium. For the gram-negative strains, *S. enterica* showed the highest susceptibility, with inhibition beginning at 20 µg/mL and reaching 40 mm at 500 µg/mL, making it the most affected strain overall. *E. coli* exhibited mild inhibition, starting at 100 µg/mL with a zone of 2 mm and increasing to 8.5 mm at 500 µg/mL. Conversely, *P. aeruginosa* showed minimal inhibition across all concentrations, with a maximum of 5 mm zone at 500 µg/mL, reflecting its inherent resistance. In summary, the n-hexane fraction displayed moderate antibacterial activity, with its strongest effects observed against *S. enterica* and gram-positive bacteria such as *S. aureus* and *B. subtilis*. Its activity was notably weaker compared to the ethyl acetate fraction, particularly against resistant strains like *P. aeruginosa*. Zones of inhibition of *n*-hexane fraction of *A. fumigatus* are given in the Figure 2.

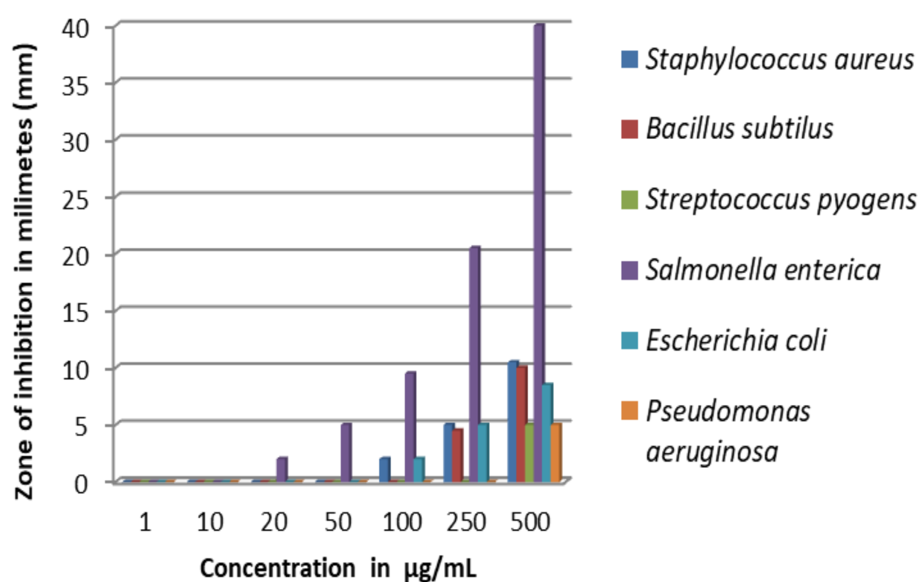

Fig. 2. Antibacterial activity of *n*-hexane fraction of *A. fumigatus*.

The antibacterial potential of both the ethyl acetate and n-hexane fractions of *P. chrysogenum* was evaluated against the test bacterial strains and the zones of inhibition (measured in millimetres) demonstrated that the ethyl acetate fraction exhibited significantly stronger antibacterial activity compared to the n-hexane fraction. For gram-positive bacteria, the ethyl acetate fraction showed strong activity, with *B. subtilis* exhibiting the largest inhibition zone (28 mm at 500 µg/mL), followed by *S. aureus* (23 mm) and *S. pyogenes* (16 mm). In contrast, the n-hexane fraction demonstrated weaker activity, with *S. aureus* showing a maximum inhibition zone of 7 mm at 500 µg/mL, while *B. subtilis* and *S. pyogenes* showed no measurable inhibition at any concentration. The antibacterial activity of ethyl acetate fraction of *P. chrysogenum* against the test bacterial strains is given in Figure 3.

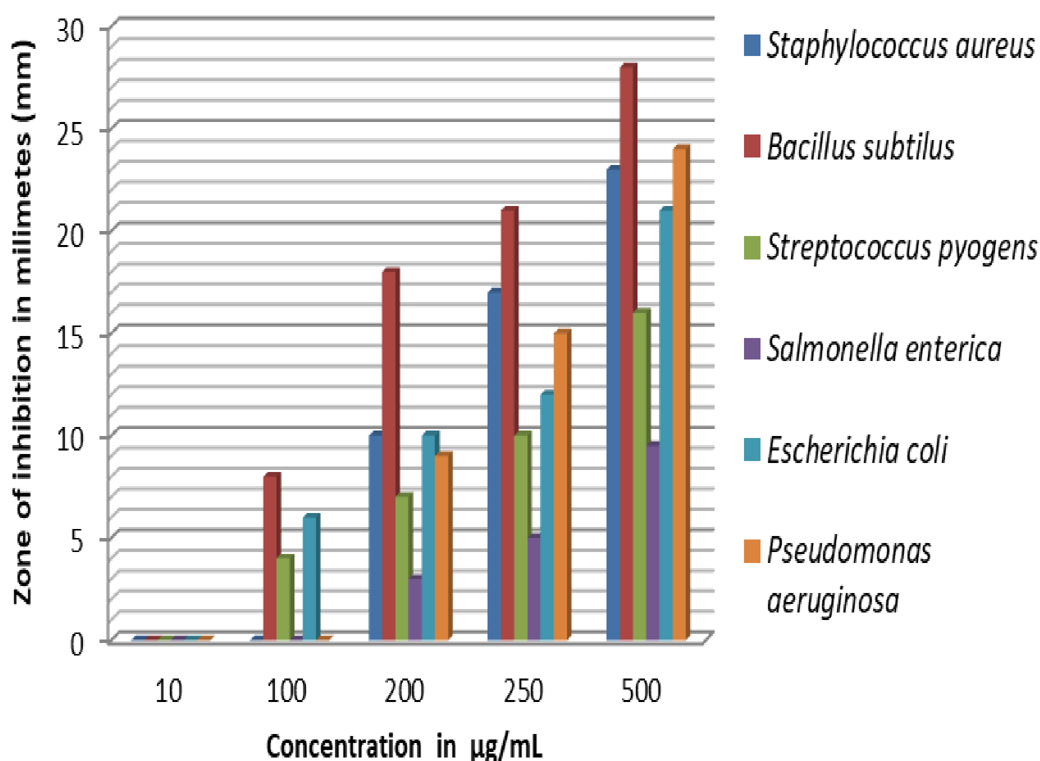

Figure 3. Antibacterial activity of crude ethylacetate fraction of *P. chrysogenum against the test bacterial stains.*

Among the gram-negative bacteria, the ethyl acetate fraction also demonstrated stronger activity, with *P. aeruginosa* showing the highest inhibition (24 mm at 500 µg/mL), followed by *E. coli* (21 mm) and *S. enterica* (9.5 mm). The n-hexane fraction exhibited moderate activity against gram-negative bacteria, with *S. enterica* showing the highest susceptibility (9.5 mm at 500 µg/mL) and *E. coli* displaying a maximum inhibition of 9 mm at the same concentration. However, *P. aeruginosa* exhibited minimal inhibition with the n-hexane fraction, with a small zone of 2.5 mm at 100 µg/mL and no activity at higher concentrations. In summary, the ethyl acetate fraction of *P. chrysogenum* displayed stronger and broader antibacterial activity compared to the n-hexane fraction, particularly against gram-positive bacteria such as *B. subtilis* and *S. aureus*. The n-hexane fraction of *Penicillium* showed limited activity, with its highest efficacy observed against gram-negative bacteria like *S. enterica* and *E. coli* (Figure 4).

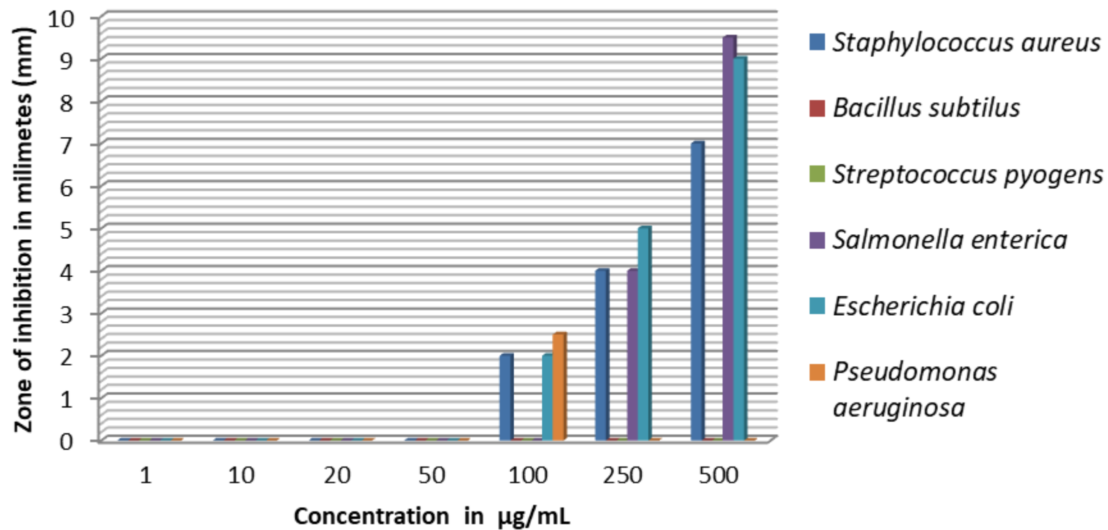

Fig 4. Antibacterial activity of n- hexane fraction of *P. chrysogenum against the test bacterial stains.*

3.2.2 Antifungal activity

The antifungal activity of the crude ethyl acetate and n-hexane fractions of *A. fumigatus* and *P. chrysogenum* was evaluated against six pathogenic fungal strains, including three yeast species (*Candida tropicalis*, *Candida albicans*, and *Rhodotorula*) and three mycelial species (*Fusarium oxysporum*, *Microsporum canis*, and *Trichophyton rubrum*). The percent inhibition, measured at concentrations ranging from 50–1000 µg/mL, demonstrated dose-dependent inhibition, with significant variation in efficacy across fungal strains.

The ethyl acetate fraction of *A. fumigatus* exhibited robust antifungal activity as shown in Figure 5. Among the yeast species, *C. albicans* showed moderate susceptibility, with percent inhibition increasing from 4% at 100 µg/mL to 35% at 1000 µg/mL. *Rhodotorula* displayed the highest inhibition among yeasts, with inhibition ranging from 2% at 50 µg/mL to 52% at 1000 µg/mL. In contrast, *C. tropicalis* exhibited the lowest susceptibility, with significant inhibition observed only at 1000 µg/mL, where the inhibition reached 27%. The mycelial fungi were more sensitive to this fraction. *M. canis* was the most affected, with inhibition increasing from 18% at 100 µg/mL to a maximum of 60.5% at 1000 µg/mL. *T. rubrum* also showed strong inhibition, ranging from 5% at 50 µg/mL to 54% at 1000 µg/mL. *F. oxysporum* demonstrated moderate susceptibility, with inhibition increasing from 4% at 100 µg/mL to 32% at 1000 µg/mL. The n-hexane fraction of *A. fumigatus* showed varying degrees of antifungal activity against the same fungal strains (Figure 6). Among the yeasts, *C. albicans* and *Rhodotorula* exhibited moderate susceptibility at higher concentrations. The percent inhibition for *C. albicans* increased from 2.5% at 250 µg/mL to 14% at 1000 µg/mL, while *Rhodotorula* exhibited the highest inhibition among yeasts, with inhibition ranging from 18% at 500 µg/mL to 37% at 1000 µg/mL. *C. tropicalis* showed minimal activity, with inhibition observed only at 1000 µg/mL (6%). Among the mycelial fungi, *M. canis* showed the highest

inhibition, increasing from 5.5% at 100 µg/mL to 45% at 1000 µg/mL. Similarly, *F. oxysporum* exhibited strong susceptibility, with inhibition increasing from 11.5% at 100 µg/mL to 40.5% at 1000 µg/mL. *T. rubrum* exhibited the least inhibition, with a maximum of 7.5% at 1000 µg/mL, indicating lower susceptibility.

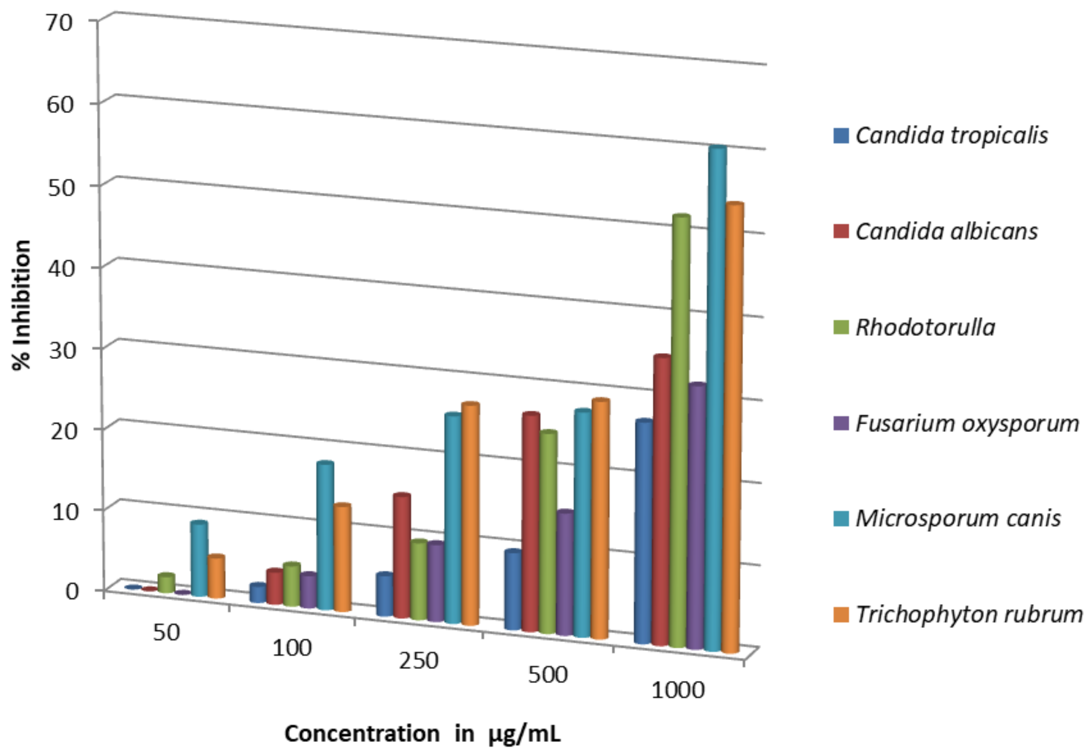

Fig. 5. Antifungal activity of ethyl acetate fraction of *A. fumigatus*

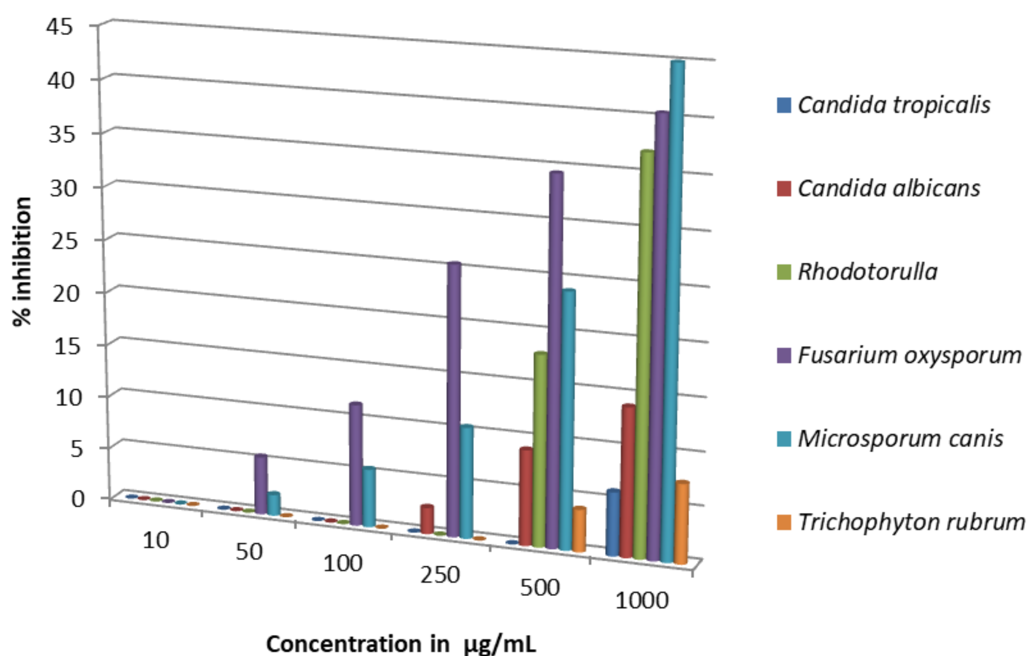

Fig. 6. Antifungal activity of n-hexane fraction of *A. fumigatus*

The ethyl acetate fraction of *P. chrysogenum* displayed potent antifungal activity in a concentration-dependent manner as given in Figure 7. Among the yeast species, *C. tropicalis* exhibited significant inhibition at higher concentrations, with inhibition increasing from 8.5% at 500 µg/mL to 44.5% at 1000 µg/mL. *C. albicans* showed a maximum inhibition of 35% at 1000 µg/mL, indicating substantial susceptibility. *Rhodotorula* exhibited the highest inhibition among the yeasts, with inhibition ranging from 18.5% at 500 µg/mL to 52% at 1000 µg/mL. The mycelial fungi were more sensitive to the ethyl acetate fraction of *P. chrysogenum* than the yeast species. *T. rubrum* was the most affected, with the highest inhibition of 70% at 1000 µg/mL, followed by *M. canis*, with inhibition increasing from 31% at 500 µg/mL to 63.5% at 1000 µg/mL. *F. oxysporum* exhibited moderate susceptibility, with inhibition increasing from 10.5% at 500 µg/mL to 38.5% at 1000 µg/mL. The results showed that the ethyl acetate fraction of *P. chrysogenum* displayed broad-spectrum antifungal activity, with particularly strong effects on mycelial fungi such as *T. rubrum* and *M. canis*. Among the yeasts, *Rhodotorula* showed the highest susceptibility, followed by *C. albicans* and *C. tropicalis*.

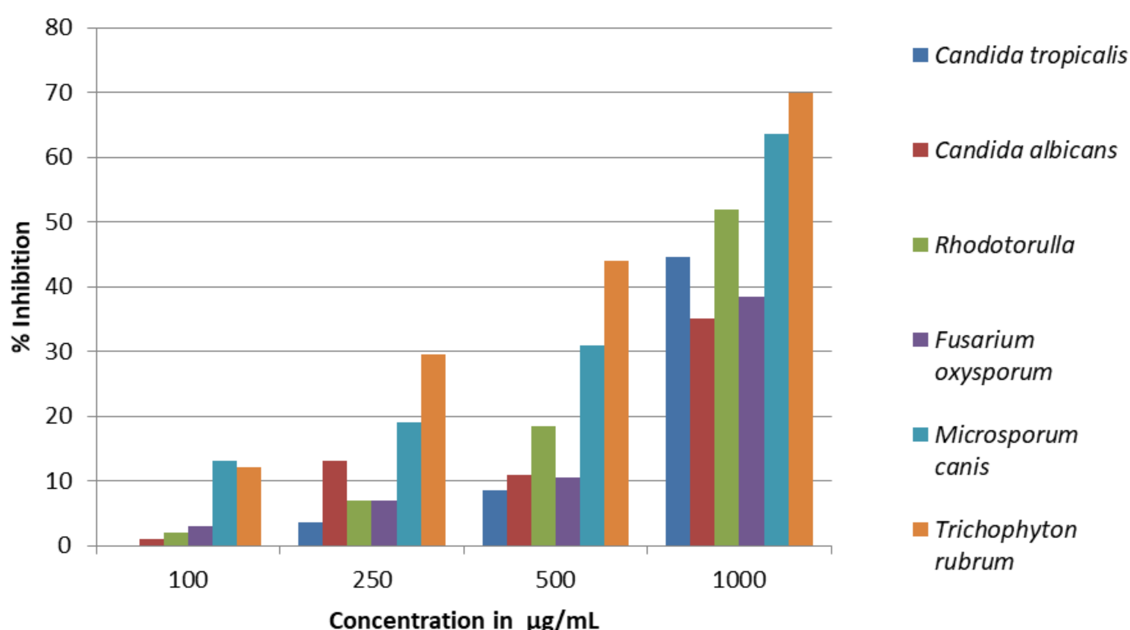

Fig. 7. Antifungal activity of ethyl acetate fraction of *P. chrysogenum*

The n-hexane fraction of *P. chrysogenum* exhibited antifungal activity only at higher concentrations of 500 µg/mL and 1000 µg/mL, as no inhibition was observed at lower concentrations (Figure 8). Among the yeast species, *C. albicans* and *Rhodotorula* displayed moderate susceptibility to this fraction, with inhibition of 22.5% and 28.5%, respectively, at 1000 µg/mL, while minimal activity was recorded at 500 µg/mL (5% for *C. albicans* and 7% for *Rhodotorula*). *C. tropicalis* exhibited the least susceptibility, with inhibition of only 6% at 1000 µg/mL and no activity at 500 µg/mL. The mycelial fungi demonstrated limited inhibition. *F. oxysporum* showed moderate susceptibility, with inhibition of 7% at 500 µg/mL and 26.5% at 1000 µg/mL. *M. canis* exhibited minor inhibition, with a zone of 6% observed only at 1000 µg/mL. *T. rubrum*, however, showed no inhibition at either concentration, indicating complete resistance to this

fraction. These results show that the ethyl acetate fractions of both *A. fumigatus* and *P. chrysogenum* exhibited stronger and broader antifungal activity compared to their respective n-hexane fractions. Among the n-hexane fractions, the activity was limited and selective, with higher susceptibility observed in yeast species such as *Rhodotorula* and *C. albicans*, while *T. rubrum* was resistant across all tested concentrations.

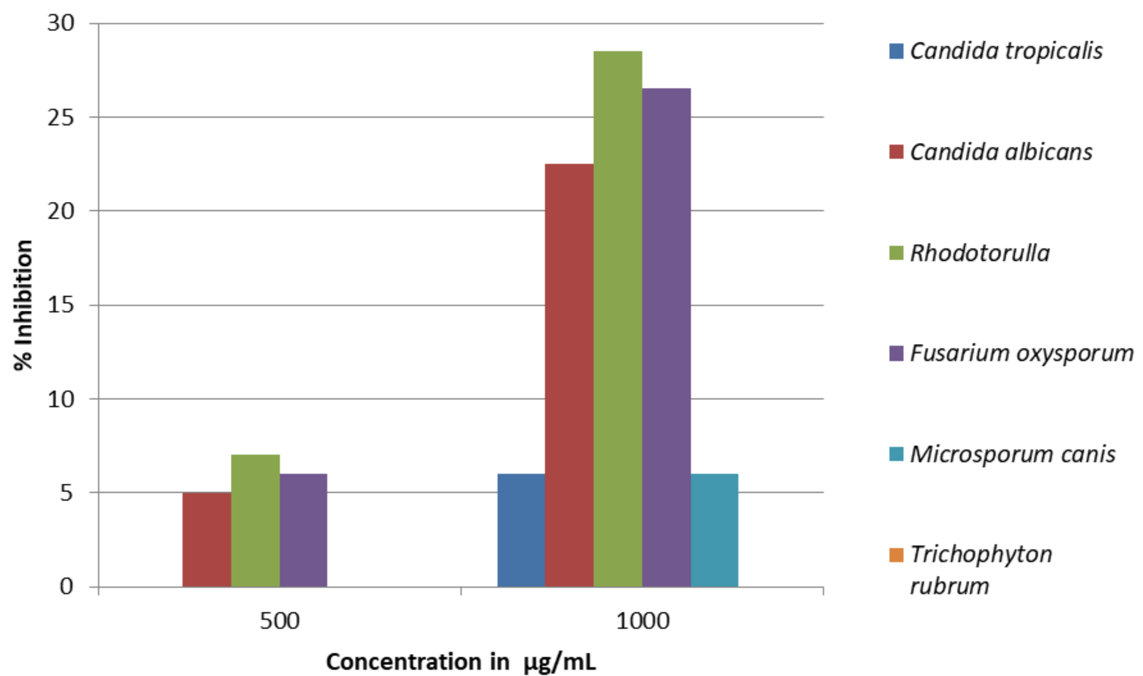

Fig 8. Antifungal activity of n-hexane fraction of *P. chrysogenum*

3.2.3 Cytotoxicity analysis using Artemia salina (Brine Shrimps)

The cytotoxic potential of both the ethyl acetate and n-hexane fractions of *A. fumigatus* and *P. chrysogenum* was evaluated using the brine shrimp lethality assay. Mortality percentages were recorded at dose concentrations of 10, 100, and 1000 µg/mL for each fraction (Figures 9 and 10). For the ethyl acetate fraction of *A. fumigatus*, mortality rates were observed to increase in a dose-dependent manner, with 3.33% mortality at 10 µg/mL, 46.66% at 100 µg/mL and 71.6% at 1000 µg/mL. Similarly, its n-hexane fraction exhibited increasing cytotoxicity, with mortality percentages of 18%, 38.33%, and 53.33% at the same concentrations. The ethyl acetate fraction of *P. chrysogenum* also demonstrated dose-dependent cytotoxicity, resulting in mortality rates of 5%, 26.66%, and 46.66% at 10, 100, and 1000 µg/mL, respectively. Notably, the n-hexane fraction of *P. chrysogenum* showed stronger cytotoxic activity, with mortality percentages of 6.66%, 56.66%, and 83.33% at the corresponding concentrations. These findings indicate that both fractions from *A. fumigatus* and *P. chrysogenum* possess cytotoxic properties, with the n-hexane fraction of *P. chrysogenum* demonstrating the highest mortality rate (83.33%) against brine shrimp at 1000 µg/mL, suggesting a higher cytotoxic potential compared to the other fractions.

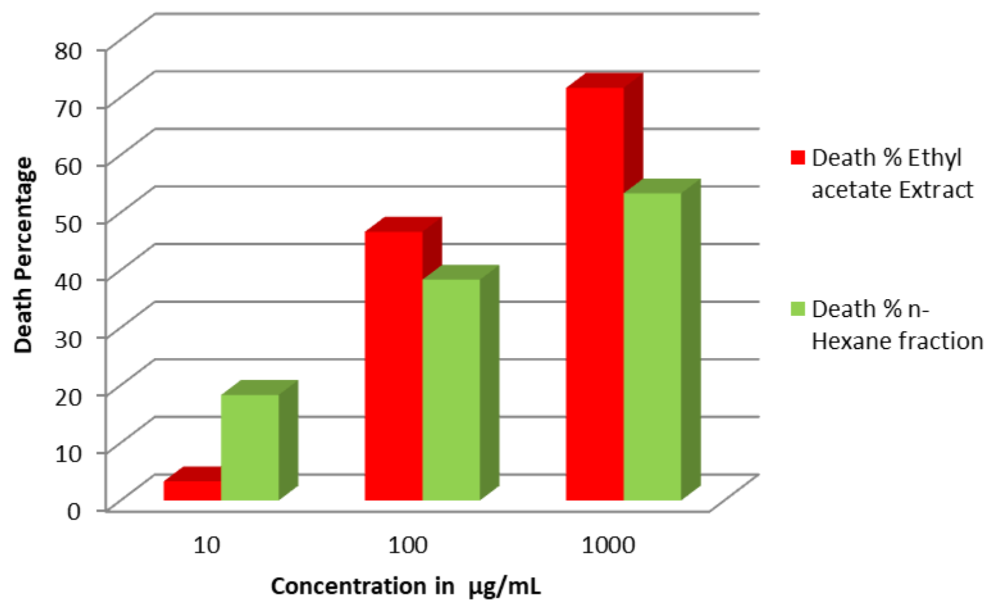

Fig 9. Cytotoxic effect of ethyl acetate and *n*-hexane fraction of *A. fumigatus*

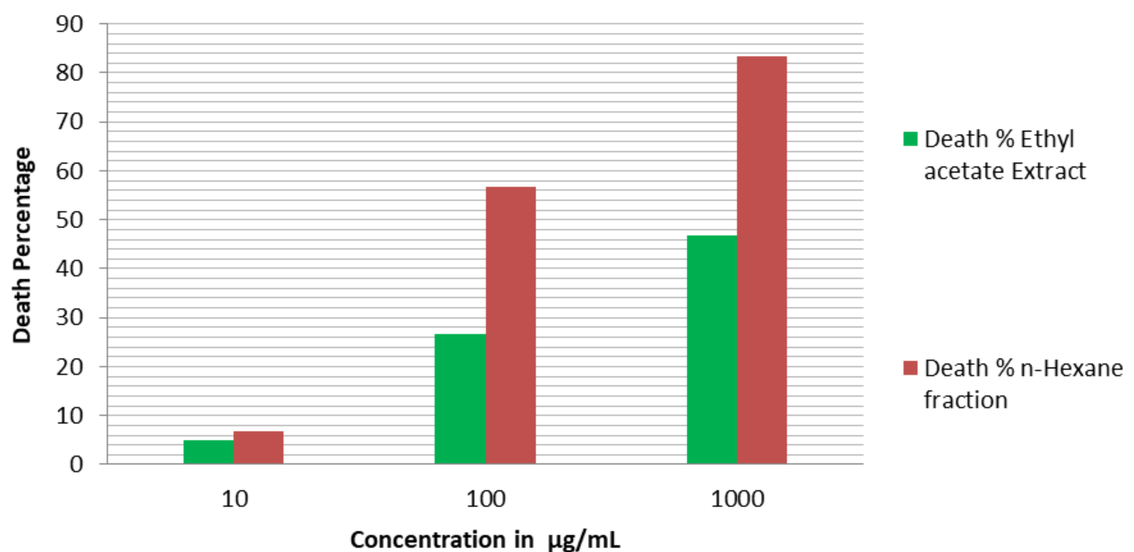

Fig 10. Cytotoxic effect of ethyl acetate and *n*-hexane fraction of *P. chrysogenum*

4. DISCUSSION

Soil fungi, particularly those from the genera *Aspergillus* and *Penicillium*, are widely recognized for their ability to produce a vast array of bioactive secondary metabolites. These metabolites, which serve as chemical defenses in their natural environment, have proven to be invaluable in the discovery of antibacterial, antifungal and cytotoxic secondary metabolites [28, 29]. In the present study, the antibacterial, antifungal and cytotoxic activities of ethyl acetate (EtoAc) and n-hexane (n-Hex) fractions from soil derived *A. fumigatus* and *P. chrysogenum* were evaluated, revealing promising bioactivity across multiple assays.

The findings align with and contribute to the growing body of literature highlighting the potential of fungal metabolites obtained from soil dwelling fungal species in pharmaceutical applications.

The antibacterial activity observed in this study was particularly notable for the EtoAc fractions, which exhibited stronger activity against gram-positive bacteria, including *S. aureus* and *B. subtilis*. These results are consistent with previous studies reporting that EtoAc fractions of fungal extracts are highly effective against gram-positive pathogens due to their ability to extract polar metabolites such as polyketides and β-lactams [30,31]. For example, the discovery of penicillin, which also targets gram-positive bacteria, emphasizes the relevance of *P. chrysogenum* in antimicrobial research. Similarly, *A. fumigatus* has been reported to produce antibacterial compounds like fumigillin [32] and the results of this study confirm its significant antibacterial potential. In comparison, the n-Hex fractions displayed limited antibacterial activity, particularly against gram-negative bacteria such as *Salmonella enterica* and *Escherichia coli*, which are known to possess intrinsic resistance due to their outer membrane. These findings are comparable to recent studies where n-Hex fractions primarily extracted hydrophobic metabolites with selective activity, such as fatty acids and terpenoids, which have shown efficacy against specific bacterial strains.

The antifungal activity demonstrated by the EtoAc fractions was even more striking, particularly against mycelial fungi such as *Trichophyton rubrum* and *Microsporum canis*. The 70% inhibition of *T. rubrum* observed for the EtoAc fraction of *P. chrysogenum* mirrors findings in recent literature, where metabolites like griseofulvin and patulin extracted with EtoAc exhibited similar efficacy [33,34]. In contrast, the n-Hex fractions showed selective activity, with notable inhibition against *F. oxysporum* and *M. canis*. These results are consistent with previous studies indicating that hydrophobic compounds extracted by n-Hex, such as sterols and lipids, play a role in disrupting fungal cell membranes [35,36]. The selective antifungal activity of the n-Hex fractions suggests their utility in targeting specific fungal pathogens, although their efficacy was lower than that of the EtoAc fractions.

The selective activity observed in this study, where the fungal extracts were more effective against gram-positive bacteria than gram-negative bacteria, can be explained by the structural differences between these bacterial groups. Gram-positive bacteria have a relatively simpler cell wall structure with a thick peptidoglycan layer, which is more accessible to certain bioactive compounds. In contrast, gram-negative bacteria possess an additional outer membrane made of lipopolysaccharides, which acts as a strong barrier, often preventing many antimicrobial agents from reaching their targets within the cell. Additionally, the specific nature of the fungal metabolites in the crude extracts may have a natural affinity for the structures or processes unique to gram-positive bacteria, such as interactions with their membrane components or cell wall synthesis pathways. Similar patterns have been observed in other studies where fungal metabolites like fusaric acid and iturin demonstrated stronger effects on gram-positive bacteria [37]. These findings emphasize the importance of further exploring and isolating the specific compounds responsible for the observed activity. Identifying the mechanisms by which these compounds interact with

bacterial cells could help refine their use as targeted antimicrobial agents and possibly expand their efficacy against a broader range of bacterial species.

Cytotoxicity assays revealed dose-dependent lethality for all fractions, with the n-Hex fraction of *P. chrysogenum* exhibiting the highest mortality against *A. salina* nauplii (83.33% at 1000 µg/mL). This is consistent with prior studies where n-Hex extracts enriched in lipophilic terpenoids demonstrated potent cytotoxic activity [38,39]. The EtoAc fractions also showed significant cytotoxicity, with mortality rates exceeding 70% for *A. fumigatus*. These results align with previous findings on cytotoxic compounds like gliotoxin and fumagillin, which are often extracted using EtoAc [40,41]. The strong cytotoxic potential observed in this study suggests the presence of secondary metabolites with therapeutic relevance, comparable to well-studied fungal metabolites like paclitaxel and myriocin, which have been developed into anticancer and immunosuppressive drugs [42].

When compared to recent literature, the bioactivity of the fractions tested in this study highlights their therapeutic potential. For instance, the antibacterial efficacy of the EtoAc fractions parallels findings where fungal metabolites exhibited activity comparable to commercial antibiotics like ciprofloxacin [43]. The antifungal activity against *T. rubrum* and *M. canis* is comparable to that of commercial antifungals such as miconazole, emphasizing the potential of these fungal metabolites to serve as alternative therapeutic agents [44]. Furthermore, the cytotoxicity results are consistent with reports of fungal metabolites being investigated as leads for anticancer therapies, demonstrating comparable lethality to chemotherapeutic agents in similar assays [45].

The findings of this study reinforce the critical role of solvent extraction methods, particularly EtoAc and n-Hex, in isolating biologically active metabolites. EtoAc fraction's ability to recover polar and moderately polar compounds was instrumental in achieving broad-spectrum activity, while n-Hex selectively extracted lipophilic compounds with targeted effects. This complementary solvent approach maximized the recovery of bioactive compounds and facilitated the identification of fractions with significant therapeutic potential. In conclusion, the results of this study align with and expand upon existing literature, demonstrating the effectiveness of *A. fumigatus* and *P. chrysogenum* in producing bioactive secondary metabolites. The findings validate the use of bioassay-guided screening and solvent-based extraction methods as reliable strategies for identifying compounds with antibacterial, antifungal and cytotoxic properties. Further purification and characterization of these metabolites could lead to the discovery of novel therapeutic agents, addressing critical challenges such as antimicrobial resistance and cancer treatment.

5. CONCLUSION

In conclusion, the bioassay-guided screening of *A. fumigatus* and *P. chrysogenum* fractions demonstrated significant antibacterial, antifungal and cytotoxic activities, with ethyl acetate fractions showing superior broad-spectrum bioactivity compared to n-hexane fractions. The findings highlight the potential of these soil fungi as prolific producers of bioactive metabolites, warranting further purification and

characterization to identify novel therapeutic compounds with applications in combating infectious diseases and cancer.

ACKNOWLEDGMENTS

The author extends heartfelt gratitude to the Centre of Biotechnology and Microbiology (COBAM), University of Peshawar, for the invaluable support in conducting this research.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest in publication of this manuscript.

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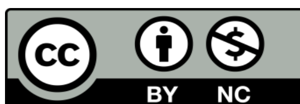

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