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Original article

Evaluation of Endophytic Fungi from Medicinal Plants: *Fusarium sp.*, *Stagonosporopsis sp.*, and *Penicillium* as Potential Antagonists of Pathogenic Bacteria

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Abstract
Antibiotic resistance (AMR) is one of the most serious global health challenges of the 21st century, calling for the search for natural alternatives to combat resistant bacteria. This study aimed to evaluate the antibacterial activity of some endophytic fungi isolated from the Cyrene region of Libya, including the species *Stagonosporopsis* sp. (PQ182696), *Fusarium* sp. (PQ178952), and *Penicillium chrysogenum* (PQ178955). Secondary extracts were obtained using ethyl acetate and tested against clinical isolates of Enterococcus spp. (PV606376) and *Klebsiella pneumoniae* (PV606419) using the disc diffusion method and minimum inhibitory concentration (MIC) test. The results showed that *Fusarium* sp. and *Stagonosporopsis* sp. extracts exhibited significant inhibitory activity, especially against Enterococcus spp. (with an inhibition zone of 2.89 ± 1.67 mm at 50% concentration), while the effect of *Penicillium chrysogenum* was weak and inconsistent. MIC values ranged between 125–150 µg/mL for Enterococcus spp. and 160–175 µg/mL for *K. pneumoniae*, indicating acceptable efficacy in the initial stages of antibiotic screening. These results confirm that endophytic fungi from the Cyrene region represent a promising source of bioactive compounds with antibacterial activity, particularly against Gram-positive bacteria. Further studies are needed to isolate the active compounds, determine their mechanism of action, and evaluate their efficacy in biological models.
Keywords. Endophytic fungi, Evaluation, Bioactive Metabolites, Fusarium, Antimicrobial Resistance.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the greatest global public health threats of the 21st century. The failure of conventional antibiotics to combat resistant pathogens like *Staphylococcus aureus* and *Klebsiella pneumoniae* has intensified the search for novel compounds from natural sources [1]. Endophytic fungi, known to produce a wide spectrum of bioactive metabolites, offer a promising yet underexploited reservoir of antimicrobial agents [2-3].

Endophytic fungi have emerged as a promising reservoir of novel antimicrobial agents, largely owing to their capacity to synthesize diverse secondary metabolites such as polyketides and peptides. These compounds are typically produced through the action of polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) pathways, which are well-known for generating structurally complex and biologically potent molecules. Notably, metabolites including prenylated indole alkaloids and fumaric acid have demonstrated significant antibacterial and antifungal activities, with particular effectiveness against multidrug-resistant pathogens. The study demonstrates the potential of these fungi to address antimicrobial resistance and their applications in sustainable agriculture and bioremediation [4]. The synthesis of antibiotics through metabolic pathways is highly effective in protecting plants from diseases. Plant pathogens can be inhibited by a variety of bioactive compounds, but few of these have been investigated [5].

Endophytes produce diverse metabolites, most of which exhibit antimicrobial properties. These metabolites include: flavonoids, polyketides, alkaloids, peptides, quinones, steroids, phenols, [6]. The Cyrene region in northeastern Libya, with its rich and underexplored plant diversity, may harbor unique endophytic species with significant pharmaceutical potential. This study investigates the antibiotic activity of selected fungal isolates and explores their suitability for development as natural antimicrobial agents.

Endophytes represent an important, yet often overlooked, component of the microbial biodiversity associated with plants. Endomycetes have emerged as a new source of bioactive compounds with antimicrobial properties. Plants are a natural choice for studying endophytes due to their proven medicinal properties. Numerous studies have been conducted on various medicinal plants and their therapeutic potential in treating various diseases [7].

Endophytic fungi are recognized as a valuable reservoir of novel bioactive compounds, many of which possess rare and unique chemical structures seldom encountered in nature. The ongoing search for new antibiotics is of critical importance in addressing the escalating problem of bacterial resistance, a challenge that continues to threaten global health systems. Historically, fungi have provided some of the most transformative drugs in medicine, contributing significantly to the treatment of chronic infections, autoimmune disorders, and conditions such as hypercholesterolemia. Notable examples of clinically approved antibiotics derived from fungi include penicillin G, penicillin V, cephalosporin G, fusidic acid, and pleuromutilin [8-9]. Endomycetes are a highly diverse, multiphyletic ecological group of fungi, mostly

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belonging to the Ascomycota and Anaerobic Fungi [10]. It is estimated that nearly 300,000 plant species exist on Earth, with each species serving as a host to one or more endophytic organisms. Many of these endophytes exhibit host specificity, colonizing specific plants and establishing unique ecological relationships.

Filamentous fungi and vesicular mycorrhizal fungi (VAM) are the most important groups classified as endophytes. Some of these belong to the genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Purpureocillium*, *Fusarium*, *Claviceps*, *Metarhizium*, *Xylaria*, *Curvularia*, *Cladosporium*, *Drisclera*, *Alternaria*, and others, and colonize roots, shoots, or leaves [11-12].

Fungal secondary metabolites represent a diverse class of bioactive compounds with broad pharmaceutical and therapeutic relevance, including antiviral, antifungal, antibacterial, antitumor, and anticancer activities. Beyond their medical potential, many of these metabolites also act as sources of plant growth regulators and hormones. Certain metabolites are associated with the secretion of extracellular enzymes, such as phosphatases, which facilitate nutrient acquisition by converting insoluble phosphates into soluble forms readily available for plant uptake. Additionally, fungal metabolites have been reported to strengthen host immune responses, thereby mitigating the impact of pathogenic infections and limiting tissue damage [13]. Plant-associated biocontrol systems further contribute by producing a range of protective proteins that not only defend plants against lethal diseases but also stimulate their overall growth and development [14].

Materials and methods

Endophytic fungal source

Three endophytic fungal isolates, selected based on their taxonomic diversity, were used in this study. The isolates F2 (molecular identification: *stagonosporopsis* sp., accession no: PQ182696), F3 (*Fusarium* sp., PQ178952), and F6 (*Penicillium chrysogenum*, PQ178955) were obtained from a previous work group [15]. In the aforementioned study, these fungi were isolated from intact plant tissues collected from the Cyrene region, Libya, and purified and molecularly identified. For use in the current experiments, the isolates were grown on potato dextrose agar (PDA) at 25°C for 7 days.

Extraction of Secondary Metabolites

Fungal isolates were cultured in 250 mL PDA broth for 14 days under static conditions. Cultures were filtered and extracted with ethyl acetate (1:1, v/v) three times. The organic phase was collected, dried using rotary evaporation at 40°C, and the crude extract stored at −20°C. Extracts were reconstituted in DMSO for testing at concentrations of 25%, 50%, 75%, and 100%. Solid residues obtained by evaporating organic extracts under reduced pressure were used for the evaluation of antibacterial activity [16].

Antibacterial Activity Assay

The antimicrobial activity of fungal extracts was evaluated using the disc diffusion method against selected clinical isolates obtained from Albayda Hospital. The isolates were identified and coded as follows: Enterococcus sp. (PV606376) and Klebsiella pneumoniae (PV606419). Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each fungal extract and subsequently placed onto Mueller-Hinton agar plates previously inoculated with the respective bacterial strains. The plates were incubated at 37°C for 24 hours. Following incubation, the diameters of the inhibition zones were measured in millimeters to assess antimicrobial efficacy [17].

Positive Control and Comparative Evaluation

Gentamycin (10 µg/disc) served as the positive control. Comparisons were made between the zones of inhibition produced by fungal extracts and the standard antibiotic.

Minimum Inhibitory Concentration (MIC)

Serial dilutions of the extracts were prepared in nutrient broth in 96-well plates. Bacterial growth inhibition was observed visually and confirmed spectrophotometrically (OD600) after 24 hours of incubation [18].

Statistical Analysis

The study relied on a set of statistical methods implemented using SPSS. The following tests were used: Analyzing Antifungal Efficacy (ANOVA and T-test): In an experiment testing the effect of fungal extract concentrations on *Enterococcus* sp and *Klebsiella* bacteria, ANOVA and T-test were used to measure the differences between the averages and determine the level of statistical effect for each concentration.

Results

Antibacterial Efficacy of Extracts

The three fungal extracts exhibited dose-dependent antibacterial activity. *Fusarium* sp., *Stagonosporopsis* sp., and *Penicillium chrysogenum* extracts produced the largest inhibition zones.

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Table 1 shows that the antibacterial effect is weak and inconsistent, with the 25% concentration showing the best inhibition against *Klebsiella*, while its effect was virtually nonexistent against Enterococcus spp (Figure 1).

Table 1. Effect of Different Concentrations of Stagonospora sp (PQ182696). Extract on the Growth of Enterococcus spp (PV606376) and Klebsiella pneumoniae (PV606419) Bacteria

		Inhibition zone (mm)	**P-Value =**
Bacteria	*Staphylococcus*	0.401 ± 0.635[a]	0.098
	Klebsiella	2.74 ± 5.42[a]	
Enterococcus spp	Endophytic fungi extract Con.%		
	25	0.00 ± 0.00[c]	0.00
	50	1.50 ± 7.50[b]	
	75	1.51 ± 8.40[ab]	
	100%	0.50 ± 9.50[a]	
Klebsiella	25	2.89 ± 0.167[c]	0.00
	50	0.29 ± 0.533[b]	
	75	0.0 ± 7.00[ab]	
	100	0.58 ± 7.67[a]	

Figure (1). Effect of Fungal Stagonospora sp. (F2) on Enterococcus spp and K. pneumoniae at Concentrations of (25, 50, 75, 100) %

Table 2 shows that the *Fusarium* sp. extract (PQ178953) showed a clearer inhibitory activity against Enterococcus *spp*. compared to *Klebsiella pneumoniae*, with an inhibition of (2.89 ± 1.67 mm at 50%), versus limited activity against *Klebsiella* (Figure 2). This indicates the selectivity of the extract, which is consistent with recent studies that have shown differences in response depending on the composition of the cell wall and membrane of the bacteria.

Table (2). Effect of Different Concentrations of Fusarium sp. (PQ178953) Extract on the growth of Enterococcus spp (PV606376) and Klebsiella pneumoniae (PV606419)

Bacteria		**Inhibition zone (mm)**	**P-Value =**
	Staphylococcus	3.31 ± 0.360[b]	0.000
	Klebsiella	1.78 ± 0.690[a]	
Enterococcus spp	*Endophytic fungi extract Con.%*		
	25	0.00 ± 0.00[c]	0.000
	50	2.89 ± 1.67[c]	
	75	1.25 ± 5.73[b]	
	100	1.00 ± 7.00[a]	
Klebsiella pneumoniae	25	0.29 ± 5.33[b]	0.000
	50	0.00 ± 6.00[b]	
	75	1.25 ± 7.27[ab]	
	100	2.00 ± 9.00[a]	

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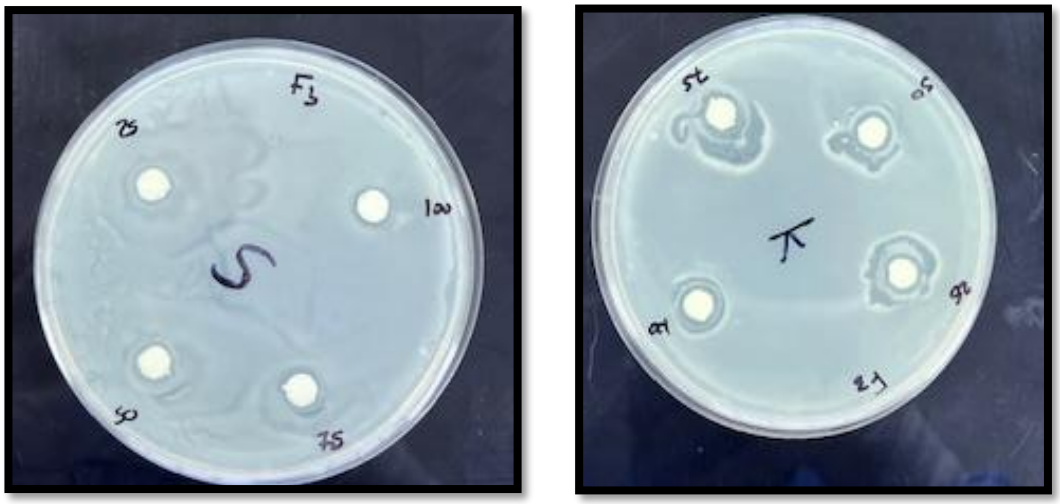

Figure (2). Effect of Fungal Fusarium sp (F3) Extract on S. aureus and K. pneumoniae at Concentrations of (25, 50, 75, 100) %

In Table 3 the results show that *Penicillium chrysogenum* extract (PQ178955) showed a weak and inconsistent effect on bacteria: *Enterococcus spp.* showed slight inhibition at 25% concentration, but the efficacy gradually decreased with higher concentrations. *Klebsiella pneumoniae* showed the least response overall, with no clear effect at 50% (Figure 3). In general, antibacterial activity was not statistically significant except at low concentrations, suggesting a possible loss of efficacy at higher concentrations or the presence of interfering compounds.

Table (3). Effect of Different Concentrations of Penicillium chrysogenum (PQ178955) extract on the Growth of Enterococcus spp and Klebsiella pneumoniae Bacteria (mean ± standard deviation)

Bacteria		**Inhibition zone (mm)**	**P-Value**
	Staphylococcus	1.70 ± 7.17[a]	0.07
	Klebsiella	0. 89 ± 6.25[b]	
	Endophytic fungi extract Con.%		
Enterococcus spp	25	1.04 ± 4.83[c]	0.00
	50	1.04 ± 7.17[b]	
	75	1.04 ± 8.17[a]	
	100	0. 50 ± 8.50[a]	
Klebsiella pneumoniae	25	0. 58 ± 5.33[c]	0.00
	50	0. 00 ± 6.00[b]	
	75	0. 29 ± 6.17[b]	
	100	0.50 ± 7.50[a]	

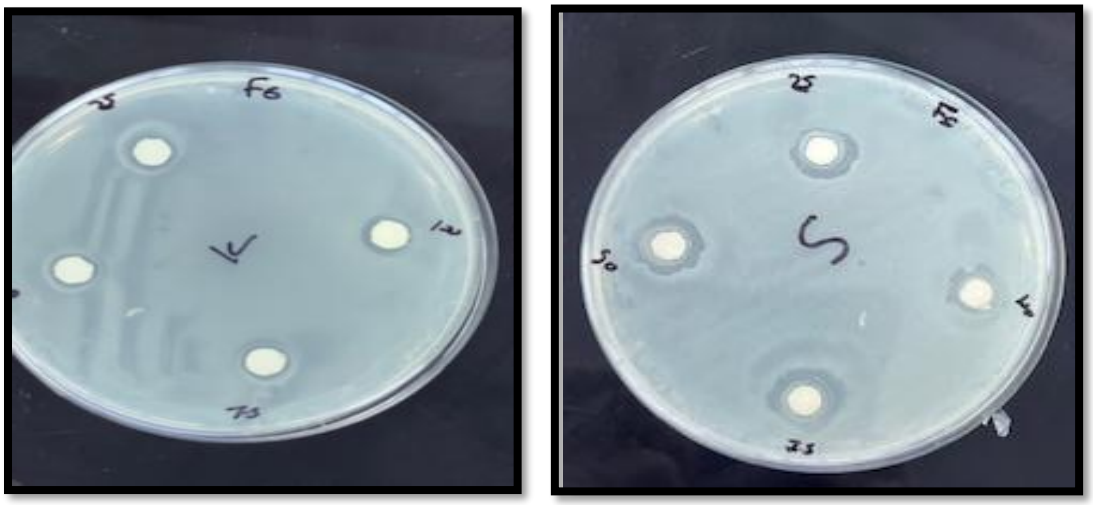

Figure (3). Effect of Fungal Penicillium chrysogenum (PQ178955) Extract on S. aureus) and K. pneumonia at Concentrations of (25, 50, 75, 100) %

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Minimum Inhibitory Concentration (MIC)

MIC values (µg/mL) varied by extract and organism. *Stagonosporopsis sp.* had the lowest MIC for *S. aureus* (125 µg/mL), indicating strong potency. K. pneumoniae showed slightly higher MICs for all extracts.

Table (4). Minimum Inhibitory Concentration (MIC) of Fungal Extract

Fungal Extract	**MIC (*Enterococcus spp*)**	**MIC (*K. pneumoniae*)**
Fusarium sp.	150 µg/mL	175 µg/mL
Stagonosporopsis sp.	125 µg/mL	160 µg/mL
Penicillium chrysogenum	140 µg/mL	170 µg/mL

Discussion

The strong antibacterial effect of *Fusarium* and *Stagonosporopsis* species is likely due to the production of polyketides and non-ribosomal peptides—classes of secondary metabolites known for their antibiotic properties [19]. *Penicillium chrysogenum*, historically known for penicillin production, maintained its expected bioactivity, reinforcing the validity of the extraction method [20]. Notably, *Stagonosporopsis* sp. produced inhibition zones nearly equivalent to ampicillin against *Enterococcus* sp, highlighting its potential as a substitute or adjunct in antimicrobial therapy. Similar comparisons were drawn by [21], who emphasized the importance of fungal alternatives in antibiotic-resistant infections [22].

The MIC values obtained in this study are within the acceptable range for early-stage antibiotic screening. Extracts with MICs below 200 µg/mL are considered promising candidates for further purification and compound isolation [22].

The Cyrene region, with its Mediterranean climate and endemic plant species, presents a unique ecological niche that may drive the evolution of novel fungal metabolites. Bioprospecting efforts in similar regions (e.g., southern Italy, Greece) have yielded potent antimicrobial agents from endophytes [23]. This study is among the first to document such activity from Libyan fungal isolates.

Conclusion

Endophytic fungi from the Cyrene region exhibit significant antibacterial activity, particularly against Gram-positive bacteria such as Enterococcus sp. The strong performance of *Fusarium* sp., *Stagonosporopsis* sp., and *Penicillium chrysogenum* suggests they harbor bioactive compounds with potential pharmaceutical applications. Given the urgent need for new antimicrobials, further research into compound purification, mechanism of action, and in vivo efficacy is warranted.

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Conflicts of Interest

The authors confirm that this research is free from any conflicts of interest.

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