MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Exploring the Antimicrobial and Antioxidant Potential of Endophytic Fungi Isolated from *Launaea mucronata*

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Received:20/9/2025
Accepted:13/10/2025

Abstract:Endophytic fungi are promising sources of bioactive secondary metabolites with potential pharmaceutical applications. This study aimed to explore the antimicrobial and antioxidant activities of endophytic fungi isolated from *Launaea mucronata* collected from coastal and inland desert habitats of Egypt. A total of seven fungal taxa were identified, belonging to the genera *Alternaria*, *Fusarium*, *Aspergillus*, and *Penicillium*. Tissue- and habitat-specific distributions were observed, with *Fusarium oxysporum* occurring in both habitats, suggesting its role as a core endophyte. Methanolic extracts of selected isolates were evaluated for antioxidant activity using the DPPH radical scavenging assay and for antimicrobial activity using the agar disk diffusion method against drug-resistant bacterial pathogens and *Candida albicans*. Results showed that *F. oxysporum* exhibited the strongest antioxidant activity (IC_{50} = 255.29 µg L^{-1}), followed by *Aspergillus flavus* (IC_{50} = 284.01 µg L^{-1}) and *Penicillium chrysogenum* (IC_{50} = 330.04 µg L^{-1}), although all were less potent than ascorbic acid (IC_{50} = 198.35 µg L^{-1}). Antimicrobial screening revealed that *F. oxysporum* had the broadest inhibitory spectrum, particularly against *Bacillus cereus* (16.8 mm) and *Escherichia coli* (12.5 mm), while *A. flavus* showed the strongest antifungal activity against *C. albicans* (17.2 mm). Gram-positive bacteria were generally more susceptible than Gram-negative strains. The findings highlight the ecological and biotechnological significance of endophytic fungi associated with *L. mucronata*, suggesting their potential as sources of novel antimicrobial and antioxidant agents.

keywords: Launaea mucronata; endophytic fungi; antimicrobial activity; DPPH assay.

1. Introduction

Medicinal plants are valuable sources of bioactive compounds with therapeutic potential. The genus *Launaea* (family Asteraceae) is traditionally used across Africa, the Mediterranean, and Asia for its anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective activities [1,2]. *Launaea mucronata* (Forssk.), a perennial herb adapted to arid and saline habitats, is particularly rich in secondary metabolites such as flavonoids, sesquiterpene lactones, terpenoids, and phenolic compounds, which underpin its pharmacological uses [3].

Phytochemical studies confirm its notable antioxidant and antimicrobial potential. El-Gendy et al. [2] identified farnesyl acetone, farnesol, and n-eicosane in the essential oil of *L. mucronata*, correlating with strong activity in DPPH, ABTS, and β-carotene assays. Likewise, Abdel-Rahman et al. [4] demonstrated that its methanolic extract inhibited *Candida albicans* and *Proteus vulgaris* while showing antioxidant activity nearly comparable to ascorbic acid. These results highlight not only the medicinal importance of *L. mucronata* but also the potential role of its associated microorganisms in contributing to, or enhancing, these bioactivities..

Endophytic fungi are microorganisms that colonize internal plant tissues without causing visible disease symptoms [5]. They are increasingly recognized as prolific producers of bioactive secondary metabolites, many of

which display antimicrobial, antioxidant, anticancer, and anti-inflammatory properties [6]. Importantly, endophytes can sometimes synthesize the same or structurally related metabolites as their host plants, but often they produce novel compounds that are absent from the plant itself [7]. This makes endophytic fungi an attractive and sustainable resource for the discovery of new natural products.

The search for alternative antimicrobial and antioxidant agents is particularly urgent due to the global rise of antimicrobial resistance (AMR) and the increasing burden of oxidative stress–related diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [8,9]. Fungal endophytes isolated from medicinal plants represent a promising but still underexplored niche in this context. By cultivating endophytic fungi under controlled conditions and screening their extracts, researchers can identify strains that produce potent bioactive metabolites suitable for drug discovery [10].

Despite the well-documented medicinal importance of *L. mucronata*, little attention has been directed toward its endophytic mycobiota. Given the bioactivity of the plant itself, it is plausible that its fungal endophytes harbor equal or even greater antimicrobial and antioxidant potential. Studies on related medicinal plants have already revealed novel metabolites of pharmaceutical importance from their endophytes [11]. Therefore, isolating, identifying, and screening endophytic fungi from *L. mucronata* could contribute significantly to the search for new antimicrobial and antioxidant agents.

The present study aims to address this knowledge gap by exploring the antimicrobial and antioxidant potential of endophytic fungi isolated from *Launaea mucronata*. Specifically, the objectives are to (i) isolate and characterize endophytic fungi associated with *L. mucronata*, (ii) evaluate their antimicrobial activity against selected bacterial and fungal pathogens, and (iii) assess their antioxidant potential *in vitro* assays.

2. Materials and Methods

2.1. Plant collection

Healthy specimens of *Launaea mucronata* (Forssk.) were collected during the flowering season from the coastal desert region of Egypt in March 2024. The plant was identified based on morphological features using standard taxonomic keys, and the identification was confirmed according to Boulos [1]. A voucher specimen (Mans. 001012013005) was prepared and deposited in the herbarium of Botany Department, Faculty of Science for future reference.

To ensure sample integrity, plants were collected from unpolluted sites and handled with sterile gloves and tools to minimize contamination. Fresh tissues (leaves, stems, and roots) were placed in sterile polyethylene bags, labeled, and immediately transported to the laboratory under cooled conditions. Samples were processed within 24 hours of collection to maintain the viability of endophytic fungi.

2.2. Isolation and Purification of Endophytic Fungi

Endophytic fungi were isolated from healthy tissues of *Launaea mucronata* (leaves, stems, and roots) following standard protocols with slight modifications [12,13]. Plant materials were first washed thoroughly under running tap water to remove debris, then rinsed with sterile distilled water. Surface sterilization was performed by sequential immersion in 70% ethanol for 1–2 minutes, followed by 2–4% sodium hypochlorite for 2–5 minutes, and a final rinse in sterile distilled water (three times) to eliminate epiphytic microorganisms [14]. To confirm sterilization efficiency, aliquots of the final rinse water were plated on potato dextrose agar (PDA) and incubated as sterility controls; the absence of microbial growth indicated successful surface sterilization.

Sterile tissues were aseptically cut into small segments (0.5–1 cm) using a sterile scalpel and placed on PDA plates supplemented with 50 mg/L chloramphenicol to suppress bacterial growth [5]. Plates were incubated at 25 ± 2 °C for 5–7 days and observed daily for fungal emergence. Hyphal tips emerging from the tissue edges were carefully transferred to fresh PDA plates to establish pure cultures.

Purification was achieved by repeated sub-culturing of single hyphal tips until morphologically uniform colonies were obtained [6]. The purified isolates were maintained on PDA slants at 4 °C for short-

term storage, while glycerol stocks (20%) were prepared and stored at –80 °C for long-term preservation [10].

2.3. Morphological Characterization

Fungal identification was performed based on colony morphology, pigmentation, and sporulation patterns. Microscopic features were examined using the needle-mount method: small fragments from sporulating regions were placed in a drop of alcohol on a clean slide, stained with lactophenol cotton blue, covered with a coverslip, and observed under a light microscope. Identification followed standard taxonomic keys [15].

2.4. Antioxidant Activity

The free radical scavenging activity of fungal extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following the method of Blois [16] with minor modifications. A 0.1 mM DPPH solution was prepared in methanol, and 1 mL of this solution was mixed with 1 mL of each extract at different concentrations (50-600 µg/L). The mixtures were incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm against a methanol blank. Ascorbic acid was used as positive control, while methanol served as a negative control. The percentage of radical scavenging activity was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH + solvent) and A_{sample} is the absorbance of the test sample. All experiments were performed in triplicate, and the concentration required to inhibit 50% of the radicals (IC_{50}) was determined from the dose–response curve [17].

2.4. Antibacterial activity

2.4.1. Tested organisms

The antimicrobial potential of fungal extracts was assessed using the agar disk diffusion (cup plate) method, performed in triplicate [18]. The test microorganisms included clinically relevant drug-resistant human pathogens: Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*), and the fungal strain *Candida albicans*. All strains were maintained in 50% glycerol stocks at –20 °C and sub-cultured monthly on appropriate media at 37 °C for 24 h to maintain viability.

For antibacterial screening, bacterial cultures were grown in Luria–Bertani (LB) broth [19] for 24 h, adjusted to a density of 10^6 CFU/mL, and spread-plated (100 µL) onto solid LB agar plates. Sterile filter paper disks (6 mm, Whatman no. 4) were impregnated with 10 µL of each extract and placed equidistantly on the inoculated agar surface. Disks containing 10% DMSO served as negative controls, while standard antibiotics (ampicillin, cefotaxime [10 µg/mL], azithromycin, tetracycline, and amphotericin B) were used as positive controls. Plates were incubated at 37 °C for 24 h in a BOD incubator, after which antimicrobial activity was determined by measuring the diameter of the inhibition zones around each disk. Experiments were carried out in a completely randomized design (CRD) with three replicates for each treatment.

3. Results and Discussion

3.1. Isolation and Purification of Endophytic Fungi

Endophytic fungi were isolated from apparently healthy *Launaea mucronata* samples growing in two habitats (coastal and inland area). As illustrated in Figure 1, the isolation was performed on four components (root, stem, leaf, and flower) of two fresh plant samples.

Endophytic fungi isolated from *Launaea mucronata* showed clear variation in both tissue preference and habitat. Seven fungal taxa were identified, mainly from the genera *Alternaria, Aspergillus, Fusarium, and Penicillium* (Figure 2). These groups are widely recognized as common endophytes of medicinal and halophytic plants, often associated with the production of antimicrobial and antioxidant metabolites [5,6].

Tissue specificity was evident, as some fungi colonized only particular plant parts. For instance, *Alternaria alternata* was consistently recovered from roots in both habitats, suggesting strong adaptation to root tissues. *Fusarium oxysporum* was detected in flowers of coastal samples and in stems and flowers of inland plants, indicating broader tissue colonization. Meanwhile, species such as

Penicillium janthinellum and Alternaria brassicicola appeared exclusively in coastal leaves and stems, respectively. This organ-specific distribution supports earlier findings that different plant tissues provide unique ecological niches for endophytes [20,10].

Habitat differences also influenced fungal diversity. Coastal plants hosted five species compared with four inland samples, with limited overlap. *F. oxysporum* was the only fungus common to both habitats, suggesting it may represent a core endophyte of *L. mucronata*. Such variation between habitats is consistent with reports that salinity, soil properties, and microclimatic conditions strongly affect fungal communities [21].

The recovery of *Penicillium, Alternaria, Fusarium,* and *Aspergillus* is noteworthy, as these genera are known producers of bioactive compounds. For example, *P. chrysogenum* is the original source of penicillin, while Alternaria spp. yield diverse polyketides with antimicrobial potential [22]. The occurrence of these fungi in *L. mucronata* suggests that they may contribute to, or even enhance, the plant's reported antimicrobial and antioxidant properties [4,2].

In summary, the endophytic community of *L. mucronata* demonstrates both tissue- and habitat-specific patterns, highlighting its ecological adaptability and potential as a source of novel bioactive metabolites.

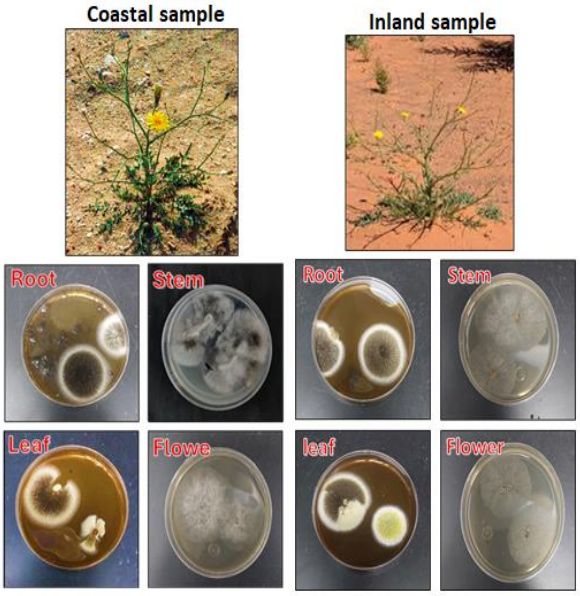

Figure 1. Isolation of endophytic fungi from *Launaea mucronata* collected from coastal habitats.

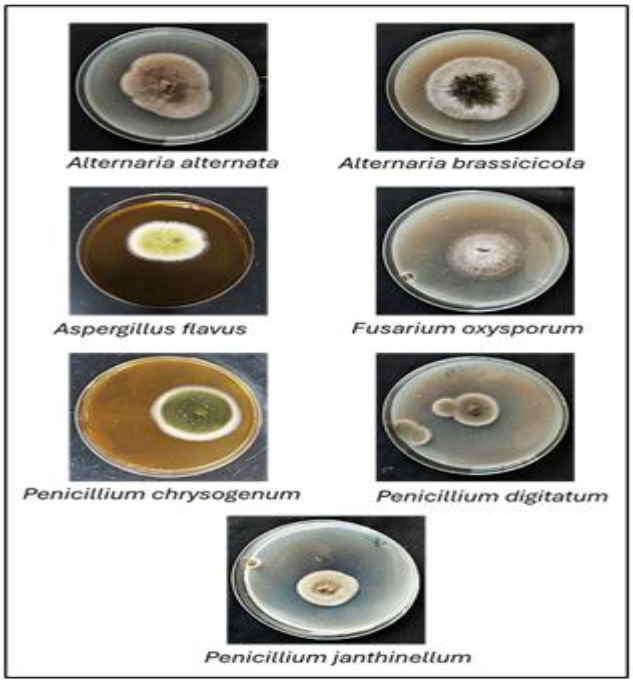

Figure 2. Purification of endophytic fungi isolated from *Launaea mucronata* in coastal and inland desert habitats.

Table : Number of fungi endophytes isolated from *Launaea mucronata* collected from different habitats

NO	Fungus name	Coastal sample				Inland sample			
		Root	Stem	Leaf	Flower	Root	Stem	Leaf	Flower
1	*Alternaria alternata*	+	-	-	-	+	-	-	-
2	*Alternaria brassicicola*	-	+	-	-	-	-	-	-
3	*Aspergillus flavus*	-	-	-	-	-		+	-
4	*Fusarium oxysporum*	-	-	-	+	-	+	-	+
5	*Penicillium chrysogenum*	+	-	-	-	-	-	-	-
6	*Penicillium digitatum*	-	-	-	-	-	-	+	-
7	*Penicillium janthinellum*	-	-	+	-	-	-	-	-

(+) Presence, (-) Absence

2. Antioxidant Activity

The methanolic extracts of the endophytic fungi *F. oxysporum, Aspergillus flavus, and P. chrysogenum,* all isolated from *Launaea mucronata*, exhibited a pronounced and dose-dependent capacity to scavenge DPPH radicals (Figure 3). The efficacy of these extracts spanned a wide range, with *F. oxysporum* demonstrating the most potent activity. Its

effect ranged from a minimum of 13.54% at the lowest concentration (50 µg L^{-1}) to a maximum of 89.9% at 600 µg L^{-1}. *Aspergillus flavus* also showed strong activity, though slightly less potent than *F. oxysporum*, with its scavenging activity increasing from a minimum of 11.68% to a maximum of 85.2% across the same concentration range. Conversely, *P. chrysogenum* exhibited the lowest antioxidant potential among the three fungi, with its activity ranging from a baseline of 9.68% to a maximum of 79.8% (Figure 3).

This performance gradient is conclusively summarized by the IC_{50} values-the concentration required for 50% scavenging-which were calculated at 255.29, 284.01, and 330.04 µg L^{-1} for *F. oxysporum*, *A. flavus*, and *P. chrysogenum*, respectively (Figure 3). All differences were statistically robust, as indicated by the low LSD values. For context, the ascorbic acid standard (IC_{50} = 198.35 µg L^{-1}) outperformed all fungal extracts, achieving a minimum of 23.3% and a maximum that exceeded 64% at a lower concentration range, confirming its superior efficacy.

These findings align with previous reports showing that endophytes, particularly from genera such as *Fusarium, Aspergillus, and Penicillium*, are prolific producers of phenolic compounds, flavonoids, and other secondary metabolites responsible for antioxidant activity [5,10]. The superior performance of *F. oxysporum* may be attributed to its ability to synthesize diverse bioactive metabolites, including polyketides and phenolic derivatives, which are known to exhibit potent radical scavenging activity. In contrast, the lower efficiency of *P. chrysogenum* suggests species-specific variations in metabolite profiles, reflecting the influence of both genetic factors and host–endophyte interactions [21].

Overall, the data support the hypothesis that endophytic fungi associated with *L. mucronata* contribute significantly to the plant's antioxidant potential. Given their relatively low IC_{50} values and strong activity at higher concentrations, these isolates represent promising candidates for further chemical characterization and possible biotechnological applications in the development of natural antioxidant agents.

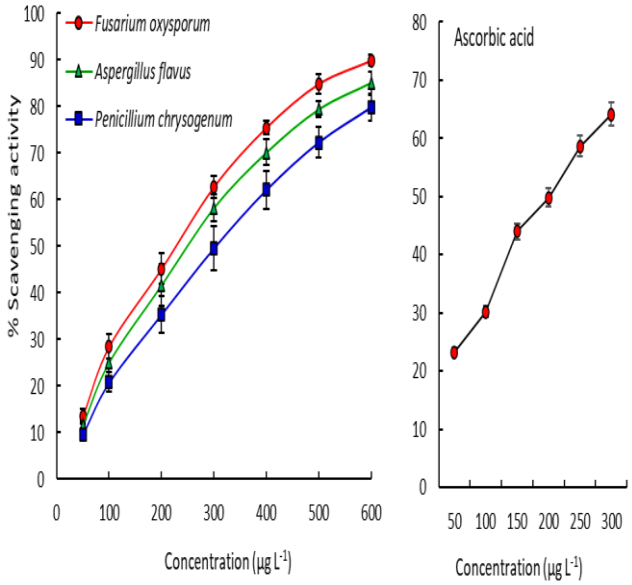

Figure 3. Scavenging activity percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) by MeOH extract of isolated from *Launaea mucronata* growing in coastal and inland desert habitats and ascorbic acid as standard.

3.3. Antibacterial activity

The methanolic extracts of endophytic fungi isolated from *Launaea mucronata* demonstrated notable antimicrobial activity, with clear variation across fungal species and microbial targets. Among the isolates, *F. oxysporum* consistently showed the strongest activity, producing inhibition zones ranging from 8.5 mm against *Pseudomonas aeruginosa* to 16.8 mm against *Bacillus cereus. Aspergillus flavus* displayed moderate activity, whereas *P. chrysogenum* generally showed the lowest inhibitory effect, with some strains such as *P. aeruginosa* and *K. pneumoniae* exhibiting no sensitivity to its extract.

A pronounced difference was observed between Gram-negative and Gram-positive bacteria. Gram-negative pathogens, particularly *P. aeruginosa* and *K. pneumoniae*, exhibited weak or no inhibition, which is consistent with their inherent resistance due to the outer membrane that restricts permeability to many bioactive compounds [23]. In contrast, Gram-positive bacteria were more susceptible, with *B. cereus* and *S. aureus* showing inhibition zones exceeding 15 mm in some cases. This pattern mirrors earlier studies indicating that Gram-positive bacteria are generally more sensitive to fungal metabolites because of their simpler cell wall architecture [5,10].

Interestingly, the fungal extracts exhibited activity against *C. albicans*, with A. flavus producing the strongest antifungal effect (17.2 mm), surpassing even *P. chrysogenum* (14.8 mm) and *F. oxysporum* (11.5 mm). Although amphotericin B (15 mm) remained a more effective antifungal agent, the activity of *A. flavus* extract suggests the presence of metabolites with potential antifungal properties comparable to commercial drugs.

When compared with standard antibiotics, fungal extracts showed lower activity overall; however, they displayed inhibitory effects against resistant strains. For example, *F. oxysporum* extract inhibited *E. coli* (12.5 mm), which was resistant to ampicillin, and *K. pneumoniae* (11.0 mm), resistant to multiple drugs. Such findings suggest that endophytic fungi may produce unique bioactive compounds capable of overcoming resistance mechanisms in certain pathogens, a feature of growing importance given the global rise of multidrug resistance [24].

Overall, the results indicate that endophytic fungi associated with *L. mucronata*, particularly *F. oxysporum* and *A. flavus*, possess promising antimicrobial potential. Their activity against both bacteria and fungi support the hypothesis that endophytes contribute to the medicinal properties of their host plant and represents a valuable source of novel antimicrobial agents for pharmaceutical development.

Table : The antimicrobial activities represented by the inhibition zone diameter (mm) of the MeOH extracts of the endophytic fungi isolated from *Launaea mucronata* growing in desert habitats and standard antibiotics.

Bacterial strain	**Fungal isolates**			**Standard antibiotics**			
	Fusarium oxysporum	*Aspergillus flavus*	*Penicillium chrysogenum*	Ampicillin	Cefotaxime	Tetracycline	Amphotericin B
Gram-negative bacteria (inhibition zone diameter (mm)							
Escherichia coli	12.5 ± 0.5	10.2 ± 0.7	8.0 ± 0.4	12 ± 1.0 (R)	18 ± 0.9 (S)	16 ± 0.8 (S)	-
Klebsiella pneumoniae	11.0 ± 0.6	9.8 ± 0.5	-	9 ± 0.5 (R)	16 ± 0.8 (S)	14 ± 0.7 (I)	-
Pseudomonas aeruginosa	8.5 ± 0.4	7.0 ± 0.3	-	- (R)	11 ± 0.6 (R)	- (R)	-
Salmonella typhi	14.1 ± 0.8	12.5 ± 0.6	10.5 ± 0.5	20 ± 1.1 (S)	23 ± 1.2 (S)	19 ± 1.0 (S)	-
Gram-positive bacteria (inhibition zone diameter (mm)							
Bacillus cereus	16.8 ± 0.9	14.3 ± 0.8	13.2 ± 0.7	28 ± 1.4 (S)	22 ± 1.1 (S)	23 ± 1.2 (S)	-
Staphylococcus aureus	15.2 ± 0.7	13.0 ± 0.6	11.5 ± 0.5	15 ± 0.9 (I)	20 ± 1.0 (S)	21 ± 1.1 (S)	-
Staphylococcus epidermidis	13.7 ± 0.5	11.8 ± 0.4	10.0 ± 0.6	40 ± 2.0 (S)	25 ± 1.3 (S)	25 ± 1.3 (S)	-
Streptococcus pneumoniae	10.0 ± 0.6	8.5 ± 0.5	-	35 ± 1.7 (S)	26 ± 1.3 (S)	27 ± 1.4 (S)	-
Fungi							
Candida albicans	11.5 ± 0.5	17.2 ± 0.9	14.8 ± 0.8	-	-	-	15 ± 0.8 (S)
$LSD_{0.05}$	**1.92*** **	**2.15*** **	**1.78*** **	**2.85*** **	**2.10*** **	**2.22** **	-

Values are average (n = 3), NA: Not active

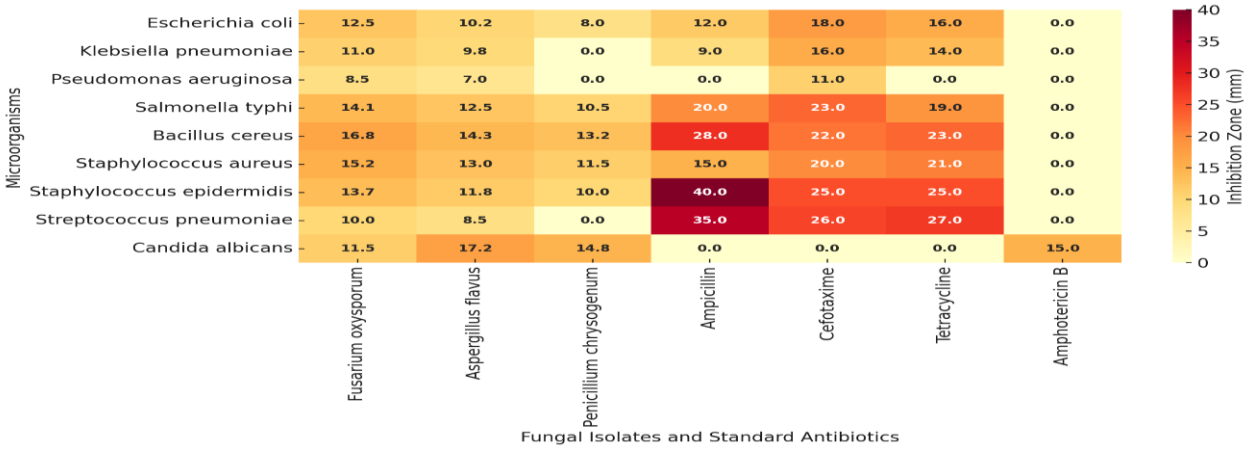

Figure 4. clustered heatmap of antimicrobial activities for the endophytic fungi isolated from *Launaea mucronata* compared.

The clustered heatmap demonstrates the antimicrobial potential of the endophytic fungal isolates (*Fusarium oxysporum*, *Aspergillus flavus*, and *Penicillium chrysogenum*) compared with standard antibiotics (Figure 4). Among the isolates, *F. oxysporum* exhibited the strongest activity, *A. flavus* showed intermediate effects, *P. chrysogenum* displayed the lowest activity, being inactive against several Gram-negative bacteria. In contrast, the standard antibiotics were generally more effective. The clustering pattern highlights a clear separation between the moderate activities of fungal extracts and the stronger effects of standard antibiotics, confirming the bioactive potential of endophytic fungi, albeit at a lower potency than conventional drugs.

4. Conclusion

This study provides new insights into the diversity and bioactivity of endophytic fungi inhabiting *Launaea mucronata*. The isolates demonstrated distinct tissue- and habitat-specific patterns, with *F. oxysporum* emerging as a dominant and bioactive species. Antioxidant assays confirmed the strong radical scavenging activity of fungal extracts, while antimicrobial screening revealed inhibitory effects against clinically relevant pathogens, including some antibiotic-resistant strains. The higher sensitivity of Gram-positive bacteria compared to Gram-negative bacteria was consistent with known structural differences in bacterial cell walls. Moreover, the antifungal activity against Candida albicans underscores the potential pharmaceutical relevance of these isolates. Collectively, the results suggest that endophytic fungi from *L. mucronata* are valuable reservoirs of bioactive compounds with prospective applications in drug discovery and natural therapeutics. Further chemical characterization and molecular studies are recommended to identify the specific metabolites responsible for these activities and to evaluate their potential for pharmaceutical development.

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