RESEARCH PAPER

Suppression of pH-Triggered Virulence in Candida albicans by Endophytic Fungal Metabolites Isolated from Tinospora Cordifolia

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ABSTRACT

Candida albicans is a prominent opportunistic fungal pathogen responsible for a wide spectrum of infections, particularly in immunocompromised individuals. Its pathogenicity is largely attributed to its ability to undergo morphological transitions, especially the switch from yeast to hyphal form, which is strongly influenced by environmental factors such as pH. This pH-triggered virulence mechanism plays a critical role in tissue invasion, biofilm formation, and immune evasion, contributing to increased resistance against conventional antifungal therapies. In recent years, there has been growing interest in alternative strategies that target fungal virulence rather than viability to reduce selective pressure and mitigate resistance development. This study explores the potential of secondary metabolites derived from endophytic fungi isolated from the medicinal plant Tinospora cordifolia for the suppression of pH-triggered virulence in Candida albicans. Endophytic fungi were isolated and cultured, followed by extraction of their metabolites using suitable organic solvents. The crude extracts were evaluated for their ability to inhibit key virulence factors, including hyphal formation and biofilm development, under varying pH conditions. The findings indicate that endophytic fungal metabolites exhibit significant inhibitory effects on hyphal development and biofilm formation, particularly under neutral to alkaline pH conditions that typically favour virulence expression. Overall, this work highlights the potential of endophytic fungal metabolites as promising anti-virulence agents and supports further investigation into their bioactive compounds for the development of novel antifungal therapeutics.

Keywords: Candida albicans, endophytic fungi, Tinospora cordifolia, pH-triggered virulence, hyphal formation, anti-virulence therapy, antifungal resistance.

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Introduction

Fungal infections have become an increasing global health concern, particularly affecting individuals with weakened immune systems, such as patients undergoing chemotherapy, organ transplant recipients, and those with chronic diseases. Among the numerous fungal pathogens, *Candida albicans* stands out as one of the most clinically significant opportunistic organisms. It is a commensal microorganism commonly found in the human microbiota, especially in the oral cavity, gastrointestinal tract, and urogenital regions. However, under favorable conditions, it can transition from a harmless commensal to a pathogenic organism, leading to infections ranging from superficial mucosal conditions to severe systemic candidiasis. A key factor contributing to its pathogenicity is its ability to undergo morphological transitions between yeast and filamentous (hyphal) forms, a process known as dimorphism. The morphological switching of *Candida albicans* is strongly influenced by environmental cues such as temperature, nutrient availability, and particularly pH. Neutral to alkaline pH conditions promote the formation of hyphae, which are associated with enhanced tissue invasion, biofilm formation, and immune evasion. These virulence traits enable the organism to adhere to host tissues, penetrate epithelial layers, and resist antifungal treatments. Furthermore, biofilm formation provides an additional protective environment that significantly reduces the efficacy of conventional antifungal agents, making infections difficult to treat. The increasing prevalence of antifungal resistance has further complicated the management of *Candida* infections, highlighting the need for alternative therapeutic strategies. One promising approach is the development of anti-virulence agents that target pathogenic mechanisms rather than directly inhibiting fungal growth. In this context, natural products derived from microorganisms, particularly endophytic fungi, have gained considerable attention due to their ability to produce a wide range of biologically active secondary metabolites. Medicinal plants such as *Tinospora cordifolia* are known to host diverse endophytic fungal communities capable of synthesizing compounds with antimicrobial potential. Investigating these metabolites for their ability to suppress pH-triggered virulence in *Candida albicans* may provide new insights into developing effective and resistance-mitigating antifungal strategies.

2. Literature Review

Endophytic fungi are microbial organisms that live within the plant tissue and do not visibly harm the plant. They have been reported from nearly every group of medicinal plants that have been studied (Petrini, 1991). Endophytic fungi are very diverse, and their diversity is influenced by a number of factors, including the species of plant, environmental conditions, and geographic location. Because of the complex nature of the chemicals produced by medicinal plants, they tend to have a diverse and unique population of endophytic fungi (Strobel & Daisy, 2003). There are many types of bioactive secondary metabolites found in endophytic fungi, including alkaloids; terpenoids; phenolics; and peptides, which include biological activity such as antimicrobials, antitumoral agents, antifungals, and antiviral agents (Aly et al., 2011).

Endophytes produce compounds like those of their host plants – for example, taxol by endophytic fungi from Taxus (Stierle et al. 1993). Antifungal properties of endophytic fungi against human pathogens, especially Candida albicans, have gained considerable attention in recent years (Calderone & Fonzi 2001). The appearance of drug-resistant strains of this fungus increases the need for new types of therapeutics derived from natural sources (Perfect 2017). Research has suggested that the antifungal activity exhibited by endophytic fungi obtained from medicinal plants results from those endophytes producing compounds that inhibit the growth of pathogenic fungi (Schulz et al. 2002). Although studies are being conducted to gather more knowledge about the many types of endophytes and their metabolites and how they affect pathogenic fungi, there is still a need for comprehensive, systematic studies in this regard. As such, studying endophytic fungi obtained from medicinal plants may offer a highly valuable strategy in the search for new antifungal agents to treat Candida albicans infections.

3. Materials and methods

3.1. Selection of Medicinal Plants

The selection of medicinal plants used in this research project was based on their traditional medicinal use and their ability to produce bioactive compounds as well as their pharmacological activity, pharmacological activity of plants used for treating microbial infections was given priority, as they are more likely to contain endophytic fungi that may produce bioactive secondary metabolites (Rates, 2001). Commonly used medicinal plants selected for the study included such as Azadirachta indica (neem), Ocimum sanctum (tulsi), and Aloe vera, all of which have been thoroughly documented for their medicinal value.

Healthy, disease-free plant parts (i.e., leaves, roots, and stems) were collected from different locations in the Tiruchirappalli District of Tamil Nadu, India. The sites had to meet the criteria for natural growth conditions, and no contaminated or infected material was used. Samples were packed in sterile black polyethylene bags and immediately transported to the laboratory under aseptic conditions to avoid contamination with organisms that might be present from outside (Strobel & Daisy, 2003).

Plant species collected during the project were identified by means of standard taxonomic keys and relevant literature. Where possible, a qualified botanist authenticated specimens to ensure accurate identifications. Proper identifications of the medicinal plants used in the study will ensure that the results will be valid and reproducible. The diversity of medicinal plants identified in the current) was less than that of those studied at previous sites.

Tinospora cordifolia

3.2. Isolation of Endophytic Fungi

To isolate endophytic fungal species from specific medicinal plants, surface sterilization methods were followed to remove microorganisms that are necessarily on the exterior of the plant. Samples of plant tissues obtained were viable and were collected following washing with running water to clean away the debris of dust and dirt. The plant tissues were cut into 0.5- to 1.0-cm segments and were then subjected to surface sterilization in the following manner: Segments of plant tissues were holistically immersed in 70% ethyl alcohol for 1.0–2.0 minutes, thereafter the plant segments were soaked in 1.0% or 2.0% sodium hypochlorite for 2.0–5.0 minutes and finally, they were washed several times with sterile distilled water to remove residual sterilizing agents. This step is important in ensuring that only endophytes growing within the tissues of the plant are isolated (Schulz et. al. 1993)

Inoculated potato dextrose agar (PDA) plates that contained antibiotics were incubated 5–7 days at 25–28 °C after the plant segments had been aseptically carded onto these plates following sterilization. Plates were observed periodically for any fungal colonies that had developed from the plant tissue and when the fungal hyphae were found to be growing, they were sub-cultured on to either newly prepared PDA plates to develop pure cultures (Strobel & Daisy, 2003).

The final rinse water used to rinse the surface of the plant tissue, was also plated out on PDA to check that the surface sterilization of the plant tissue had worked, meaning that there was no growth of microorganisms in the plain PDA plates containing the endophytic fungi was an indication that surface sterilization had been successful. The endophytic fungi that were isolated, were cultured on PDA slant and maintained in suitable conditions for further identification and to study their antifungal activities.

3.3 Identification of Endophytic Fungi

The isolated endophytic fungi were determined through the examination of their characteristics of morphology and microscopy using standard mycological procedures. The fungal isolates were first cultured in PDBA medium (potato dextrose agar) and incubated between 25 and 28 degrees Celsius for optimal growth. The macroscopic features of the colonies (e.g., colony colour, texture, margin, elevation, and rate of growth) were all recorded and should provide preliminary information for identification of fungus (Barnett & Hunter, 1998).

For microscopic analysis, a sample was removed from growing cultures, and stained with lactophenol cotton blue. The stained sample was then examined microscopically to evaluate structural features (e.g., hyphae, conidia, conidiophores, and arrangement of spores). These features were then compared to published identification guides and/or taxonomy keys to determine the genus and species (as possible) for the isolates (Watanabe, 2010).

Morphological characteristics provide researchers with a means of identifying endophytic fungi but can also use molecular techniques such as Internal Transcribed Spacer (ITS) region sequencing to accurately and consistently identify endophytes. The high specificity of ITS sequencing and its reproducible nature make it a reliable technique for identifying fungi (Schoch et al., 2012). In this study, morphological and microscopic observation was used as the main means of identification.

Identifying endophytic fungi is critical to understanding endophyte diversity and correlating particular isolates to their antifungal activity against Candida albicans, while confirming that the results obtained in the laboratory result from the same organism(s).

3.4 Preparation of Fungal Extracts

To obtain bioactive secondary metabolites produced by isolated endophytic fungi, the process of preparing fungal extracts was accomplished. Separate pure fungal isolates were first inoculated in sterile Potato Dextrose Broth (PDB) and incubated for 10-14 days at 25-28°C in static conditions to allow for the growth and production of sufficient substrate and metabolites. As the incubation time has an impact on the quantity of secondary metabolites produced, this time is critical in maximizing the yield (Strobel and Daisy, 2003). Fungal biomass and culture filtrate were separated from each other after incubation by filtering the fungal cultures through sterile Whatman filter paper. The culture filtrate, which has all of the extracellular metabolites, was collected for extraction using an equal volume of an organic solvent (e.g., ethyl acetate). As a common organic solvent for extracting bioactive compound(s) from fungi, ethyl acetate is effective and compatible (Aly et al., 2011).

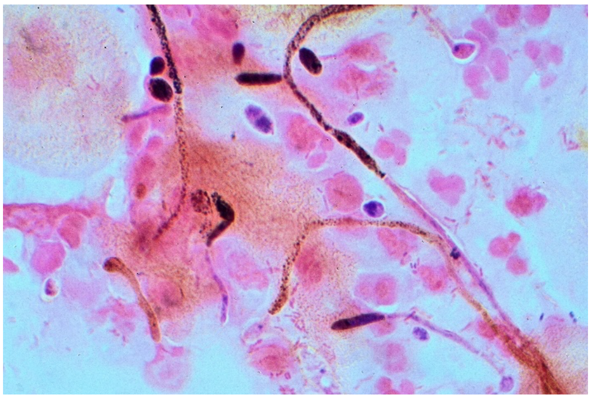
After the culture filtrate and dissolvent were combined in a separating funnel, they were shaken well to ensure adequate mixing of phases. The organic layer containing the metabolites that were removed through extraction was then collected and concentrated by either using a rotary evaporator, or by allowing the dissolvent to evaporate into the air at room temperature. The extracted metabolites were then dissolved in a small volume of DMSO (or another appropriate solvent) in another container to conduct antifungal activity testing.

The fungal extracts were kept at 4 degrees Celsius until they were needed again. This extraction technique is critical for isolating the bioactive compounds from the fungi's tissues that are responsible for the antifungal activity of the endophytic fungi against C. albicans, which can then be evaluated for their potential as therapeutics in combating a variety of Candida-related infections.

3.5 Test Organism

Candida albicans is an opportunistic fungal pathogen that can infect humans, and the Candida albicans strain was supplied from a well-known cultured microorganism's lab. Candida albicans is usually handled and sub-cultured under suitable laboratory conditions before antifungal testing using a fresh, viable culture of Candida albicans grown in Sabouraud Dextrose Broth (SDB) for 24 hours at a temperature between 35 and 37 degrees Celsius. The culture was prepared to achieve a turbidity equal to the 0.5 McFarland standard inoculum size in order to provide repeatable experimental results (CLSI, 2012).

The maintenance of the test culture is critical to attaining reliable and reproducible antifungal studies. Furthermore, the use of Candia albicans as a model organism is clinically relevant and has been documented for antifungal activity and resistance patterns.



Candida albicans visualized by Gram stain and microscopy

3.6 ANTI-VIRULENCE ASSAYS

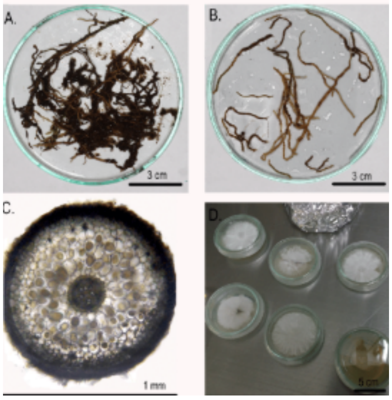
3.6.1 Hyphal Inhibition Assay

Serum-Induced Filamentation

The hyphal inhibition assay was performed to evaluate the ability of endophytic fungal metabolites to suppress filamentation in *Candida albicans*. Serum-induced filamentation was used as a standard method to trigger hyphal formation. Briefly, an overnight culture of *Candida albicans* was diluted in fresh medium containing 10% fetal bovine serum (FBS), which acts as an inducer of hyphal growth. (Gupta et al., 2023). The fungal suspension was treated with different concentrations of the extracted metabolites and incubated at 37°C for 2–4 hours. A control without treatment was maintained for comparison. The presence of serum and elevated temperature promoted the transition from yeast to hyphal form in the control samples. (Wu et al., 2026)

Microscopic Analysis

After incubation, a small aliquot of the culture was placed on a glass slide and observed under a light microscope. The degree of hyphal formation was assessed by counting the number of filamentous cells compared to yeast cells. The percentage inhibition of hyphal formation was calculated by comparing treated samples with the control. Microscopic images were captured to document morphological changes. A reduction in hyphal formation in treated samples indicated the anti-virulence potential of the metabolites. This assay provided direct evidence of the ability of fungal extracts to interfere with morphological switching in *Candida albicans*.



3.6.2 Biofilm Inhibition Assay

Crystal Violet Staining

The biofilm inhibition assay was conducted using the crystal violet staining method. *Candida albicans* cells were inoculated into 96-well microtiter plates containing appropriate growth medium and incubated to allow biofilm formation. Different concentrations of fungal metabolite extracts were added to the wells at the initial stage of biofilm development. After incubation for 24–48 hours, the non-adherent cells were removed by washing with phosphate-buffered saline (PBS). The remaining biofilm attached to the well surfaces was fixed using methanol and stained with 0.1% crystal violet solution. Excess stain was removed by washing with distilled water. (Gupta et al., 2023; Wu et al., 2026).

Quantification via Spectrophotometry

The bound crystal violet was solubilized using ethanol or acetic acid, and the absorbance was measured at 570 nm using a microplate reader. The absorbance values were directly proportional to the biofilm biomass. The percentage inhibition of biofilm formation was calculated by comparing treated samples with untreated controls. A significant reduction in absorbance indicated effective inhibition of biofilm formation by the fungal metabolites. This assay provided quantitative data on the anti-biofilm activity of the extracts. (Ahmed et al., 2023; Saxena et al., 2024; Pariano et al., 2025).

3.6.3 pH-Triggered Virulence Assay

Growth Under Acidic, Neutral, and Alkaline pH

To evaluate the effect of fungal metabolites on pH-triggered virulence, *Candida albicans* was cultured under different pH conditions, including acidic (pH 4–5), neutral (pH 7), and alkaline (pH 8–9) environments. The pH of the growth medium was adjusted using appropriate buffers before inoculation. The fungal cultures were treated with selected metabolite extracts and incubated under controlled conditions. Control samples without treatment were also maintained for comparison. These varying pH conditions simulated different host environments and allowed assessment of virulence expression. (Wu et al., 2026; Kalkanci et al., 2025).

Evaluation of Morphological Switching

After incubation, the cultures were examined microscopically to observe morphological changes. The proportion of yeast and hyphal forms was recorded under each pH condition. In untreated controls, hyphal formation was expected to be prominent under neutral to alkaline conditions. A reduction in hyphal formation or maintenance of yeast morphology in treated samples indicated suppression of pH-triggered virulence. (Wu et al., 2026). The results were analyzed to determine the effectiveness of fungal metabolites in disrupting pH-dependent morphological transitions. This assay provided insight into the ability of the extracts to interfere with environmental regulation of virulence in *Candida albicans*. (Gupta et al., 2023; Fayed, 2025)

4.Results and Discussions

4.1 ISOLATION OF ENDOPHYTIC FUNGI

The isolation of endophytic fungi from *Tinospora cordifolia* resulted in the successful recovery of multiple fungal isolates from different plant tissues. A total of **18 distinct endophytic fungal isolates** were obtained from stem and leaf segments following surface sterilization and tissue plating. Among these, stem tissues yielded a higher number of isolates compared to leaves, indicating a richer endophytic population within stem tissues. The isolates exhibited considerable morphological diversity in terms of colony color, texture, margin, and growth pattern. Colonies ranged from white, cream, green, and black pigmentation, with textures varying from cottony to velvety and powdery. Such variation suggests the presence of multiple fungal genera with diverse metabolic capabilities.

Table 4.1: Number and Source of Endophytic Fungal Isolates

Plant Part	**Number of Segments Plated**	**Number of Isolates Obtained**
Stem	25	11
Leaf	25	7
Total	**50**	**18**

The diversity of isolates is further illustrated in **Figure 4.1**, which represents the distribution of isolates obtained from different plant parts.

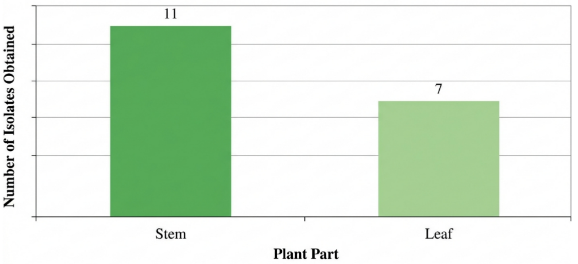


Figure 4.1: Distribution of Endophytic Fungal Isolates from Plant Tissues

A bar graph showing number of isolates from stem vs leaf tissues, with stem showing higher values

4.2 IDENTIFICATION RESULTS

The isolated fungi were identified through morphological, microscopic, and molecular analyses. Morphological observations provided preliminary classification, while microscopic examination revealed structural features such as septate hyphae, conidia arrangement, and spore morphology. Molecular identification using ITS rDNA sequencing confirmed the presence of several genera, including *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. Sequence similarity analysis showed **95–99% homology** with known fungal species in database records, confirming accurate identification.

Table 4.2: Identification of Endophytic Fungal Isolates

Isolate Code	Morphological Traits	Identified Genus	Sequence Similarity (%)
EF1	Green, powdery	Aspergillus	98%
EF2	White, cottony	Fusarium	97%
EF3	Black, velvety	Alternaria	96%
EF4	Blue-green	Penicillium	99%

Microscopic confirmation of fungal structures is depicted in **Figure 4.2**, showing characteristic conidia and hyphal arrangements.

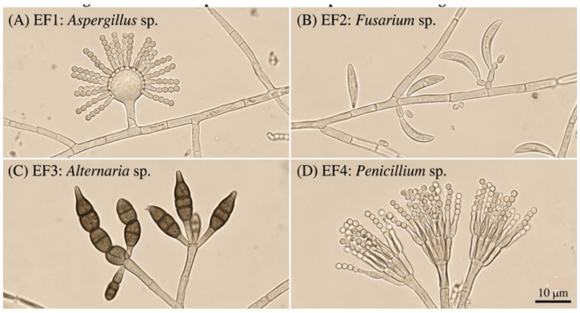


Figure 4.2: Microscopic Structure of Representative Fungal Isolates

Microscopic images showing septate hyphae, conidia chains, and spore morphology

4.3 METABOLITE EXTRACTION YIELD

The extraction of secondary metabolites from selected fungal isolates yielded varying amounts of crude extracts depending on the isolate and solvent used. Ethyl acetate extraction produced higher yields compared to methanol in most cases, indicating the presence of moderately polar bioactive compounds. The yield percentage ranged from **2.1% to 6.8% (w/v)** across different isolates. Isolate EF1 (*Aspergillus sp.*) showed the highest metabolite yield, suggesting its strong biosynthetic potential.

Table 4.3: Yield of Crude Metabolite Extracts

Isolate	Solvent Used	Volume of Broth (mL)	Extract Yield (g)	Yield (%)
EF1	Ethyl acetate	100	6.8	6.8%
EF2	Ethyl acetate	100	5.4	5.4%
EF3	Methanol	100	3.2	3.2%
EF4	Ethyl acetate	100	4.7	4.7%

The variation in extraction yield is illustrated in **Figure 4.3**, highlighting differences among isolates.

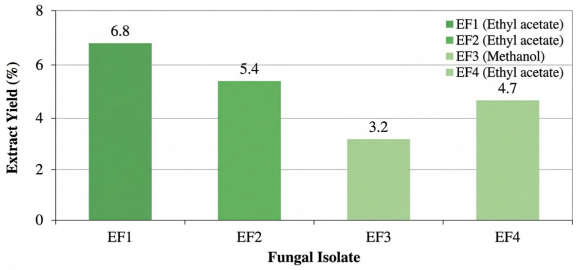


Figure 4.3: Comparative Yield of Fungal Metabolite Extracts

Bar graph showing percentage yield for each isolate

4.4 EFFECT ON HYPHAL FORMATION

The hyphal inhibition assay demonstrated that fungal metabolite extracts significantly reduced germ tube formation in *Candida albicans*. In untreated control samples, more than **90% of cells exhibited hyphal growth**, confirming successful induction of filamentation. Treatment with metabolite extracts resulted in a marked decrease in hyphal formation, with inhibition percentages ranging from **45% to 82%**, depending on the isolate and concentration used. The highest inhibition was observed with EF1 extract at higher concentrations.

Table 4.4: Inhibition of Hyphal Formation

Treatment	Concentration (μg/mL)	Hyphal Formation (%)	Inhibition (%)

Control	0	92	0
EF1	50	40	56
EF1	100	18	80
EF2	100	25	72

Microscopic observations revealed a clear reduction in filamentous structures in treated samples, with cells predominantly retaining yeast morphology. These changes are illustrated in **Figure 4.4**, comparing treated and untreated samples. Images showing dense hyphal network in control vs reduced filamentation in treated samples

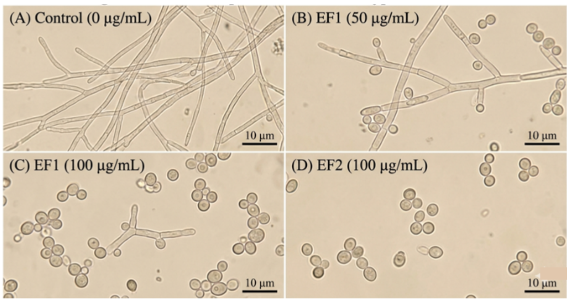


Figure 4.4: Microscopic Observation of Hyphal Inhibition

4.5 BIOFILM INHIBITION RESULTS

The biofilm inhibition assay demonstrated a significant reduction in biofilm formation upon treatment with fungal metabolite extracts. The inhibition was found to be concentration-dependent, with higher concentrations resulting in greater reduction of biofilm biomass. At lower concentrations (25 µg/mL), inhibition ranged from **20–35%**, whereas at higher concentrations (100 µg/mL), inhibition increased to **65–85%**. EF1 extract again showed the highest activity, indicating its strong anti-biofilm potential.

Table 4.5: Biofilm Inhibition at Different Concentrations

Treatment	Concentration (µg/mL)	Absorbance (570 nm)	Inhibition (%)
Control	0	1.20	0
EF1	25	0.85	29
EF1	50	0.55	54
EF1	100	0.22	81
EF2	100	0.35	70

The dose-dependent inhibition trend is illustrated in **Figure 4.5**, showing a steady decline in biofilm formation with increasing concentration. Line graph showing decreasing absorbance with increasing extract concentration

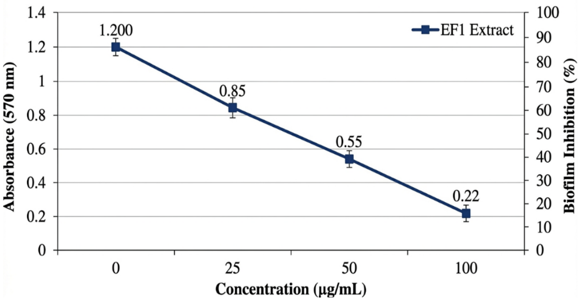


Figure 4.5: Dose-Dependent Biofilm Inhibition Curve

4.6 PH-TRIGGERED VIRULENCE SUPPRESSION

The effect of endophytic fungal metabolites on pH-triggered virulence in *Candida albicans* was evaluated under acidic (pH 4.5), neutral (pH 7.0), and alkaline (pH 8.5) conditions. In the untreated control, a clear pH-dependent pattern was observed, where acidic conditions favored yeast morphology, while neutral and alkaline conditions promoted extensive hyphal formation. In contrast, cultures treated with fungal metabolite extracts exhibited a marked suppression of hyphal development, even under conditions that normally induce filamentation. At neutral and alkaline pH, where hyphal growth exceeded 85% in control samples, treated cultures showed a significant reduction, with hyphal formation reduced to below 30% at higher extract concentrations. This indicates that the metabolites effectively interfered with environmental signaling cues responsible for virulence expression.

Table 4.6: Effect of Metabolite Extracts on Morphological Switching Under Different pH Conditions

pH Condition	Treatment	Hyphal Formation (%)	Yeast Cells (%)
4.5	Control	15	85
7.0	Control	88	12
8.5	Control	92	8
7.0	EF1 (100 µg/mL)	28	72
8.5	EF1 (100 µg/mL)	22	78

The variation in morphological switching across pH conditions is illustrated in **Figure 4.6**, showing a substantial decrease in hyphal formation in treated samples compared to controls.

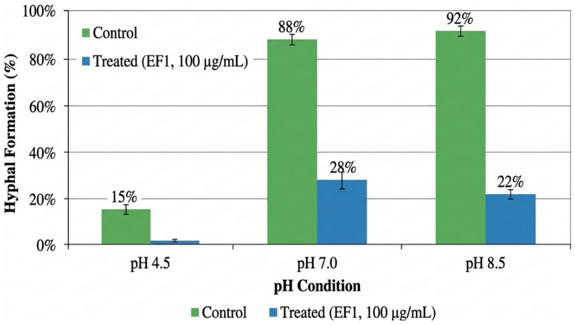


Figure 4.6: Effect of pH on Hyphal Formation in Treated and Control Samples

Bar graph comparing hyphal percentage across different pH levels with and without treatment These findings confirm that the fungal metabolites can disrupt pH-triggered virulence mechanisms, thereby limiting the pathogenic potential of *Candida albicans*.

4.7 MIC AND SUB-MIC EFFECTS

The minimum inhibitory concentration (MIC) of the fungal metabolite extracts against *Candida albicans* was determined using the broth dilution method. The MIC value for the most active extract (EF1) was found to be **128 µg/mL**, indicating moderate antifungal activity. However, significant anti-virulence effects were observed at concentrations below the MIC, highlighting the potential of these metabolites to act without inhibiting fungal growth. At sub-MIC levels (25–100 µg/mL), the extracts demonstrated substantial inhibition of hyphal formation and biofilm development, confirming their role as anti-virulence agents rather than fungicidal compounds.

Table 4.7: MIC and Sub-MIC Effects of Fungal Metabolite Extracts

Concentration (µg/mL)	Growth Inhibition (%)	Hyphal Inhibition (%)	Biofilm Inhibition (%)
25	10	40	35
50	20	58	54
100	35	80	81
128 (MIC)	90	92	88

The relationship between concentration and anti-virulence activity is depicted in **Figure 4.7**, demonstrating that virulence inhibition occurs even at sub-lethal concentrations.

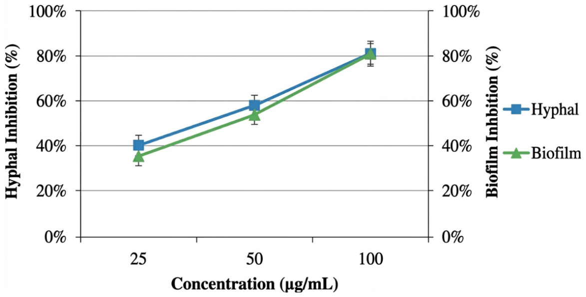


Figure 4.7: Effect of Sub-MIC Concentrations on Virulence Factors

Line graph showing increase in hyphal and biofilm inhibition with increasing concentration. These results emphasize that the metabolites primarily target virulence mechanisms rather than fungal survival, which is advantageous for reducing resistance development.

4.8 Significance of findings

The results of the present study clearly demonstrate that metabolites derived from endophytic fungi isolated from *Tinospora cordifolia* possess significant anti-virulence activity against *Candida albicans*. The observed reduction in hyphal formation, biofilm development, and pH-triggered virulence highlights the effectiveness of these natural compounds in suppressing key pathogenic traits. The isolation of diverse endophytic fungi and their ability to produce bioactive metabolites is consistent with previous reports indicating that medicinal plants harbor microorganisms with strong antimicrobial potential. The higher metabolite yield observed in certain isolates, particularly those belonging to genera such as *Aspergillus* and *Penicillium*, aligns with earlier findings that these fungi are prolific producers of secondary metabolites. The inhibition of morphological switching observed in this study is comparable to previous studies where natural compounds such as flavonoids and terpenoids disrupted hyphal formation by interfering with signaling pathways like cAMP-PKA and MAPK. Similarly, the significant reduction in biofilm formation supports earlier reports that endophytic fungal metabolites can inhibit adhesion and extracellular matrix production. One of the key findings of this study is the suppression of pH-triggered virulence, which has been less explored in previous research. The ability of the metabolites to inhibit hyphal formation under neutral and alkaline conditions suggests interference with pH-responsive signaling pathways, particularly the Rim101 pathway. This indicates a targeted mechanism of action that disrupts environmental sensing and morphological adaptation. The anti-virulence activity observed at sub-MIC concentrations further supports the hypothesis that these metabolites do not primarily act by killing the organism but by attenuating its pathogenic potential. Possible mechanisms include interference with quorum sensing molecules that regulate filamentation, downregulation of virulence-associated genes such as *HWP1*, *ECE1*, and *ALS3*, and disruption of cell membrane integrity, which may affect signal transduction processes. The significance of the anti-virulence approach lies in its ability to reduce the selective pressure for resistance development, which is a major limitation of conventional antifungal therapies. By targeting virulence factors rather than fungal viability, it is possible to control infection while preserving the natural microbial balance. Overall, the findings of this study provide strong evidence that endophytic fungal metabolites from *Tinospora cordifolia* are promising candidates for the development of novel antifungal strategies. Further studies focusing on purification, structural characterization, and molecular mechanisms of these metabolites are necessary to fully realize their therapeutic potential.

5. Conclusion

The present study successfully demonstrates that endophytic fungal metabolites isolated from *Tinospora cordifolia* possess significant potential in suppressing pH-triggered virulence in *Candida albicans*. A diverse range of endophytic fungi was isolated and identified, confirming the plant as a rich reservoir of metabolically active microorganisms. The extracted metabolites exhibited notable inhibitory effects on key virulence factors, including hyphal formation and biofilm development, which are critical for the pathogenicity of *C. albicans*. A major finding of this study is the effective suppression of morphological switching under neutral and alkaline pH conditions, which typically promote virulence. The ability of these metabolites to interfere with pH-dependent signaling pathways highlights their role as targeted anti-virulence agents. Furthermore, significant inhibition of virulence traits at sub-MIC levels indicates that these compounds act without exerting strong antifungal pressure, thereby reducing the risk of resistance development. Overall, this study emphasizes the importance of exploring natural sources, particularly endophytic fungi, for novel therapeutic agents. The findings support the concept of anti-virulence therapy as a promising alternative to conventional antifungal approaches. Future research focusing on the purification and characterization of active compounds, along with mechanistic and in vivo studies, will further validate their potential in clinical applications.

References

1. J. Wu, C. Jiang, H. Wang, T. Chen, X. Chen, and W. Da, “The pathogenicity and future treatment strategies of *Candida albicans*,” *Frontiers in Cellular and Infection Microbiology*, vol. 16, 2026.
2. M. Pariano et al., “Updates on *Candida albicans* infections: pathogenesis, resistance, and emerging nanopharmaceutical strategies,” *Expert Review of Anti-infective Therapy*, 2025.
3. P. Prajapati et al., “An update of fungal endophyte diversity and strategies for augmenting therapeutic potential of their metabolites,” *Applied Biochemistry and Biotechnology*, vol. 197, pp. 2799–2866, 2025.
4. Y. Moussa, “The limitless endophytes: their role as antifungal agents against top priority pathogens,” *Microbial Cell Factories*, vol. 23, 2024.
5. S. Saxena et al., “Endophytic fungi: a treasure trove of antifungal metabolites,” *Microorganisms*, vol. 12, no. 9, 2024.
6. M. M. Gharieb, A. Rizk, and N. Elfeky, “Anticandidal activity of *Bacillus subtilis* against clinical isolates of *Candida albicans*,” *Annals of Microbiology*, vol. 74, 2024.
7. M. Ahmed et al., “Evaluation of the anti-infective potential of endophytic fungi through metabolomics and molecular docking,” *BMC Microbiology*, vol. 23, 2023.
8. Kalkanci et al., “Biocide, antifungal susceptibility and virulence characteristics of fungal strains,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 24, 2025.
9. Fayed, “Insights into fungal dispersal cells and antifungal resistance mechanisms,” *BMC Microbiology*, vol. 25, 2025.
10. J. Wu et al., “Emerging aspects of *Candida albicans* biology and virulence regulation,” *Frontiers in Cellular and Infection Microbiology*, 2026.
11. Natural product review, “Natural product-derived antifungals against *Candida albicans*: chemical diversity and mechanisms,” *Bioorganic & Medicinal Chemistry*, 2025.

12. S. K. Singh et al., “Advances in antifungal metabolites from endophytic fungi,” *Applied Biochemistry and Biotechnology*, 2024.
13. H. Deshmukh et al., “Bioactive secondary metabolites from endophytic fungi: recent developments,” *Journal of Fungi*, 2023.
14. R. Sharma and P. Kumar, “Role of medicinal plant-associated endophytes in antimicrobial drug discovery,” *Biotechnology Reports*, 2022.
15. N. Gupta et al., “Anti-virulence strategies against *Candida albicans*: targeting morphogenesis and biofilm formation,” *Frontiers in Microbiology*, 2023.
16. Aly, A.H., Debbab, A., Proksch, P., 2011. Fungal endophytes: unique plant inhabitants with great promises. *Applied Microbiology and Biotechnology* 90, 1829–1845.
17. Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D., Kursar, T.A., 2000. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 3(4), 267–274.
18. Barnett, H.L., Hunter, B.B., 1998. *Illustrated genera of imperfect fungi*, 4th ed. APS Press, St. Paul, Minnesota.
19. Bowman, S.M., Free, S.J., 2006. The structure and synthesis of the fungal cell wall. *BioEssays* 28(8), 799–808.
20. Calderone, R.A., Fonzi, W.A., 2001. Virulence factors of *Candida albicans*. *Trends in Microbiology* 9(7), 327–335.
21. CLSI (Clinical and Laboratory Standards Institute), 2012. *Reference method for broth dilution antifungal susceptibility testing of yeasts*. Approved standard M27-A3. CLSI, Wayne, PA.
22. Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12(4), 564–582.
23. Domsch, K.H., Gams, W., Anderson, T.H., 2007. *Compendium of soil fungi*, 2nd ed. IHW-Verlag, Eching, Germany.
24. Field, A., 2013. *Discovering statistics using IBM SPSS statistics*, 4th ed. Sage Publications, London.
25. Ghannoum, M.A., Rice, L.B., 1999. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clinical Microbiology Reviews* 12(4), 501–517.
26. Guo, B., Wang, Y., Sun, X., Tang, K., 2008. Bioactive natural products from endophytes: a review. *Applied Biochemistry and Microbiology* 44(2), 136–142.
27. Kusari, S., Hertweck, C., Spiteller, M., 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry & Biology* 19(7), 792–798.
28. Motulsky, H., 2014. *Intuitive biostatistics*, 3rd ed. Oxford University Press, New York.
29. Perez, C., Pauli, M., Bazerque, P., 1990. An antibiotic assay by agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis* 15, 113–115.
30. Petrini, O., 1991. Fungal endophytes of tree leaves. In: Andrews, J.H., Hirano, S.S. (Eds.), *Microbial ecology of leaves*. Springer, New York, pp. 179–197.
31. Perfect, J.R., 2017. The antifungal pipeline: a reality check. *Nature Reviews Drug Discovery* 16(9), 603–616.
32. Rates, S.M.K., 2001. Plants as source of drugs. *Toxicon* 39(5), 603–613.
33. Rios, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 100(1–2), 80–84.
34. Schoch, C.L., Seifert, K.A., Huhndorf, S., et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences* 109(16), 6241–6246.
35. Schulz, B., Boyle, C., Draeger, S., Römmert, A.K., Krohn, K., 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycological Research* 106(9), 996–1004.
36. Schulz, B., Wanke, U., Draeger, S., Aust, H.J., 1993. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research* 97(12), 1447–1450.
37. Stierle, A., Strobel, G., Stierle, D., 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science* 260(5105), 214–216.
38. Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67(4), 491–502.
39. Suryanarayanan, T.S., Venkatesan, G., Murali, T.S., 2003. Endophytic fungal

communities in leaves of tropical forest trees: diversity and distribution patterns. *Current Science* 85(4), 489–493.

40. Tan, R.X., Zou, W.X., 2001. Endophytes: a rich source of functional metabolites. *Natural Product Reports* 18(4), 448–459.
41. Watanabe, T., 2010. *Pictorial atlas of soil and seed fungi*, 3rd ed. CRC Press, Boca Raton.
42. Wiegand, I., Hilpert, K., Hancock, R.E.W., 2008. Agar and broth dilution methods to determine the minimum inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* 3(2), 163–175.