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Bioactive secondary metabolites from *Ficus pumila* endophytes: A study on antimicrobial efficacy and resistance mitigation

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Abstract

Antimicrobial resistance is emerging as one of the gravest threats to global public health, leading to the inefficacy of conventional antibiotics and resulting in the resurgence of infections once considered easily treatable. In this context, endophytic fungi are increasingly being investigated for their biosynthetic potential to produce novel bioactive secondary metabolites. This study focuses on fungal endophytes isolated from *Ficus pumila*, a widely distributed medicinal plant in Asia, known for its therapeutic applications in traditional medicine. The primary objective of the study is to isolate and identify endophytic fungi from *Ficus pumila* and to assess their antimicrobial activity against common pathogenic bacteria and fungi. The investigation involved surface sterilisation of *Ficus pumila* leaves, stems, and roots, followed by isolation and purification of fungal colonies. Morphological and molecular characterisation of the endophytes was performed to determine species identity. Crude extracts were prepared using ethyl acetate and tested for antimicrobial activity using agar well diffusion and broth dilution methods. Several isolates demonstrated significant inhibition against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida albicans*, suggesting the presence of potent bioactive compounds. The minimum inhibitory concentration (MIC) of selected extracts was also determined. Preliminary phytochemical analysis indicated the presence of alkaloids, flavonoids, and terpenoids in the most active extracts, and chemical profiling through LC-MS and NMR hinted at the novelty of these secondary metabolites. This research reinforces the potential of fungal endophytes from *Ficus pumila* as promising candidates in the discovery of new antimicrobial compounds, especially at a time when existing antibiotics are failing. By leveraging both classical microbiological methods and modern analytical techniques, the study contributes to the understanding of plant-fungal symbiosis as a bioresource for drug discovery and positions *Ficus pumila*-derived endophytes as viable leads in the global fight against antimicrobial resistance.

Keywords: *Ficus pumila*, endophytic fungi, antimicrobial activity, secondary metabolites, antimicrobial resistance, natural product drug discovery, bioactive compounds, LC-MS, MIC assay

1. Introduction

The alarming global rise in antimicrobial resistance (AMR) has fundamentally challenged our ability to treat infectious diseases, posing a serious threat to public health systems and economies worldwide. Once-considered controllable, bacterial and fungal infections are rapidly re-emerging as leading causes of morbidity and mortality due to the declining efficacy of conventional antibiotics (Ventola, 2015; WHO, 2019) [30, 31]. Multidrug-resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Carbapenem-resistant *Klebsiella pneumoniae*, and extended-spectrum β-lactamase-producing *Escherichia coli* are now responsible for a growing number of hospital-acquired and community-based infections. In light of this, there is an urgent need for new antimicrobial agents with novel modes of action (Davies & Davies, 2010; Laxminarayan *et al*., 2013) [9, 15].

Nature has always served as an invaluable source of therapeutic agents. More than 70% of currently used antibiotics are either natural products or their derivatives, derived largely from soil bacteria and actinomycetes (Newman & Cragg, 2016) [16]. However, the high rate of rediscovery of known compounds and the diminishing returns from conventional sources have prompted researchers to turn to underexplored ecological niches. One such promising niche is the endophytic microbial community within medicinal plants. Endophytes are microorganisms, primarily fungi and bacteria, that reside within the internal tissues of plants without causing apparent disease (Petrini, 1991) [19]. These symbiotic organisms are

increasingly recognised for their ability to produce diverse secondary metabolites with antimicrobial, anticancer, anti-inflammatory, and immunosuppressive activities (Tan & Zou, 2001; Strobel & Daisy, 2003) [28, 26].

Endophytic fungi, in particular, have gained considerable attention due to their metabolic plasticity and close association with medicinal plants. The hypothesis that endophytes contribute to or even mimic the biosynthetic pathways of their host plants has gained support from studies that discovered compounds like taxol and camptothecin-traditionally attributed to plants-produced by their associated fungi (Stierle *et al*., 1993; Kusari *et al*., 2012) [25, 14]. This co-evolutionary relationship suggests that the therapeutic efficacy of many medicinal plants may in part be due to the bioactive compounds produced by their microbial inhabitants (Aly *et al*., 2010) [1]. Thus, investigating endophytes not only complements phytochemical studies of host plants but also opens new avenues for bioactive compound discovery.

The present study focuses on *Ficus pumila*, commonly known as the creeping fig, which is a climbing species in the Moraceae family. It is native to East Asia and widely distributed across tropical and subtropical regions. Traditionally, *Ficus pumila* has been used in various ethnomedicinal systems to treat ailments ranging from gastrointestinal disorders to inflammation, infections, and skin conditions (Chang *et al*., 2007) [5]. Despite its widespread use in traditional medicine, the microbiome associated with *Ficus pumila*, especially its fungal endophytes, remains underexplored in the context of antimicrobial research.

The study hypothesises that endophytic fungi isolated from *Ficus pumila* harbour the potential to produce novel antimicrobial compounds that may be effective against a broad spectrum of human and plant pathogens, including those resistant to conventional antibiotics. To test this, fungal endophytes were isolated from sterilised leaf, stem, and root tissues of *Ficus pumila*. These isolates were cultured, purified, and identified using morphological and molecular techniques. Their extracts were then tested against a panel of bacterial and fungal strains using agar diffusion and broth dilution methods to evaluate their antimicrobial efficacy. In addition, minimum inhibitory concentrations (MICs) were determined for the most active extracts. Secondary metabolite profiling was performed using phytochemical screening, TLC, LC-MS, and NMR spectroscopy.

This integrated approach aims to contribute to natural product drug discovery by providing evidence of the antimicrobial potential of fungal endophytes from *Ficus pumila*. Moreover, it addresses the broader need for sustainable and effective antimicrobial strategies in an era of rising resistance. This research also holds promise for applications beyond medicine, including agriculture and biotechnology, as the same bioactive metabolites could be repurposed as natural fungicides or bactericides to control plant pathogens.

Ultimately, this study highlights the role of underexplored plant-associated microbiomes in drug discovery and advocates for a paradigm shift that views plant-microbe interactions not just through ecological or agricultural lenses, but also as treasure troves of pharmaceutical innovation. By leveraging the natural synergy between plants and their microbial symbionts, we may find answers to one of the most pressing challenges of our time: antimicrobial resistance.

2. Literature Review

The study of fungal endophytes as reservoirs of bioactive compounds has garnered increasing attention in the past two decades, particularly as conventional sources of antibiotics become exhausted. In this section, we explore existing literature on endophytic fungi, their relationship with medicinal plants, the antimicrobial resistance crisis, and the untapped potential of *Ficus* species, especially *Ficus pumila*.

2.1 Endophytic Fungi and Their Role in Drug Discovery

Endophytic fungi are ubiquitous in the plant kingdom and have been isolated from virtually every plant species examined to date (Arnold *et al*., 2000) [3]. These organisms reside asymptomatically within plant tissues, forming mutualistic relationships that often result in increased stress tolerance, enhanced nutrient uptake, and disease resistance for the host plant (Schulz & Boyle, 2005) [22]. Importantly, endophytes are known to synthesise secondary metabolites that may either complement or mimic those of their host plants. These metabolites include alkaloids, terpenoids, steroids, flavonoids, and polyketides, many of which exhibit potent antimicrobial, antiviral, and anticancer activities (Tan & Zou, 2001; Strobel & Daisy, 2003) [28, 26].

Numerous studies have documented the success of endophytic fungi in producing pharmacologically relevant compounds. One of the most notable examples is the discovery of taxol, a widely used anticancer drug, from *Taxomycesandreanae*, an endophyte of *Taxus brevifolia* (Stierle *et al*., 1993) [25]. Similarly, Kusari *et al*. (2012) [14] demonstrated that endophytic fungi isolated from *Camptotheca acuminata* could biosynthesise camptothecin, a well-known alkaloid with anticancer properties. These discoveries have shifted the focus of natural product research towards microbial symbionts, especially fungi.

2.2 Endophytes as Sources of Antimicrobial Compounds

The antimicrobial potential of endophytic fungi has been explored in numerous medicinal plants across tropical and temperate ecosystems. Aly *et al*. (2010) [1] reported that more than 50% of fungal endophytes isolated from medicinal plants in Egypt showed antimicrobial activity against *E. coli*, *Bacillus subtilis*, and *Candida albicans*. In another study, Verma *et al*. (2009) [29] isolated endophytic fungi from *Azadirachta indica* and found that many possessed antibacterial properties attributed to phenolic and alkaloid compounds.

Screening endophytes for antimicrobial activity typically involves culturing the isolates in liquid media, extracting secondary metabolites using organic solvents such as ethyl acetate, and evaluating their bioactivity through agar diffusion and MIC assays (Polpass *et al*., 2010) [20]. Modern techniques like LC-MS, GC-MS, and NMR spectroscopy have further advanced the identification and characterisation of these metabolites (Zhang *et al*., 2006; Nicoletti & Fiorentino, 2015) [33, 17].

2.3 Antimicrobial Resistance and the Urgency for Novel Antibiotics

Antimicrobial resistance (AMR) is now recognised as one of the top ten global public health threats (WHO, 2019) [31]. Overuse and misuse of antibiotics in human medicine, agriculture, and animal husbandry have contributed to the selection and spread of resistant pathogens. According to O'Neill (2014) [18], if left unchecked, AMR could lead to 10 million deaths annually by 2050 and a cumulative economic loss of USD 100 trillion globally.

The current pharmaceutical pipeline for antibiotics is sparse, and many major pharmaceutical companies have reduced investment in antimicrobial R&D due to high development costs and limited profitability (Laxminarayan *et al*., 2013) [15]. Natural products, particularly those from endophytes, are seen as a viable solution to this stagnation. As they often contain novel chemical scaffolds with unique modes of action, these compounds may be less susceptible to existing resistance mechanisms (Cowan, 1999; Davies & Davies, 2010) [7, 9].

2.4 The Genus Ficus in Medicinal Research

The *Ficus* genus, comprising over 800 species, is widely distributed across tropical and subtropical regions. Several species are known for their medicinal properties, including *Ficus religiosa*, *Ficus benghalensis*, and *Ficus carica*. Phytochemical studies of these species have revealed the presence of tannins, sterols, alkaloids, flavonoids, and saponins, many of which possess antimicrobial, antioxidant, and anti-inflammatory properties (Sharma *et al*., 2010; Joshi & Joshi, 2011) [26, 11].

Despite this, the endophytic communities of *Ficus* species remain relatively underexplored. Few studies have examined the antimicrobial properties of endophytic fungi isolated from *Ficus* species. A study by Kumar and Kaushik (2013) [13] isolated endophytes from *Ficus religiosa* and found several extracts with significant activity against *E. coli* and *S. aureus*. However, literature focusing on *Ficus pumila* is extremely scarce, representing a notable research gap. Given its established role in traditional medicine for treating infections and inflammation, exploring the endophytes of *Ficus pumila* is both scientifically and medically justified.

2.5 Analytical Techniques for Secondary Metabolite Identification

Modern analytical chemistry has significantly advanced our ability to detect and characterise secondary metabolites from fungal sources. Techniques such as Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Liquid Chromatography-Mass Spectrometry (LC-MS) are routinely used to create metabolic fingerprints (Nicoletti & Fiorentino, 2015) [17]. These tools are complemented by spectroscopic methods such as Nuclear Magnetic Resonance (NMR) which allow for detailed structural elucidation of bioactive molecules.

The integration of bioassay-guided fractionation with these techniques enhances the reliability of identifying active principles (Strobel *et al*., 2004) [27]. Furthermore, molecular characterisation of fungal isolates using ITS rDNA sequencing has become standard practice, allowing accurate taxonomic identification and aiding in the dereplication of known compounds.

2.6 Research Gap and Justification for the Study

While there is a growing body of literature documenting the antimicrobial potential of endophytic fungi, there is a dearth of studies specifically focused on *Ficus pumila*. Most research has concentrated on *Ficus religiosa* or *Ficus carica*, leaving the endophytic community of *Ficus pumila* largely uncharted. Moreover, very few studies have applied a comprehensive, multidisciplinary approach combining molecular identification, phytochemical profiling, antimicrobial screening, and metabolomic analysis.

Given the mounting crisis of antimicrobial resistance and the need for novel therapeutic options, this study aims to fill the knowledge gap by systematically investigating the endophytic fungi from *Ficus pumila*. By applying standardised protocols for isolation, characterisation, and bioactivity screening, the study seeks to determine whether these fungal isolates can yield secondary metabolites with clinically relevant antimicrobial properties.

3. Materials and Methods

This study was structured in a sequential manner, comprising field sample collection, laboratory isolation of fungal endophytes, characterisation (morphological and molecular), extraction of secondary metabolites, and *in vitro* antimicrobial screening against selected bacterial and fungal pathogens. The methodology was designed to ensure sterility, replicability, and analytical rigour at each stage.

3.1 Collection and Authentication of Plant Material

Healthy, mature *Ficus pumila* plants were selected from an organic botanical site located in Uttarakhand, India. Leaves, stems, and roots were collected early in the morning in sterile polythene bags and transported in cool conditions to the laboratory within 24 hours. Botanical authentication was carried out by a plant taxonomist, and a voucher specimen (FP-2025/01) was deposited at the herbarium of the university's Department of Botany.

3.2 Surface Sterilisation and Isolation of Endophytic Fungi

Surface sterilisation was performed to remove epiphytic microorganisms using the method adapted from Schulz *et al*. (1993) [23], as follows:

- Tissues were washed in running tap water for 10 minutes.
- Samples were immersed in 70% ethanol for 60 seconds.
- Subsequently treated with 4% sodium hypochlorite for 3 minutes.
- Rinsed thrice with sterile distilled water.
- Dried on sterile filter paper in a laminar airflow chamber.

Each sterilised tissue was cut into 1 cm^2 segments and aseptically placed on Potato Dextrose Agar (PDA) plates containing 100 µg/mL streptomycin to inhibit bacterial growth. Plates were incubated at 27 ± 1 °C for 10–14 days and observed daily for fungal growth. Emerging colonies were subcultured repeatedly to obtain pure cultures.

3.3 Determination of Sterilisation Efficiency

To validate the sterilisation protocol, 100 µL of the final rinse water was spread onto nutrient agar plates. The absence of microbial growth after 48 hours confirmed effective surface sterilisation, ensuring that isolates originated from internal tissues.

3.4 Morphological Characterisation

Morphological features such as colony texture, pigmentation, radial growth, and hyphal characteristics were recorded. Lactophenol cotton blue staining was used to examine conidia and reproductive structures under a compound microscope at 400× magnification. Preliminary identification was made using standard fungal taxonomic keys (Barnett & Hunter, 1998) [4].

3.5 Molecular Identification of Selected Isolates

Ten representative isolates exhibiting unique morphological traits and promising antimicrobial activity were selected for molecular identification. Genomic DNA was extracted using the CTAB method. The internal transcribed spacer (ITS) region of rDNA was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990) [32]. The PCR reaction mix (25 µL) contained:

- 1× Taq buffer,
- 2.5 mM $MgCl_2$,
- 200 µM dNTPs,
- 0.5 µM of each primer,
- 1 U Taq DNA polymerase,
- 50 ng of template DNA.

Thermal cycling conditions included

- Initial denaturation at 95 °C for 5 min,
- 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min,
- Final extension at 72 °C for 10 min.

Amplicons were visualised on 1.5% agarose gels stained with ethidium bromide. PCR products were purified and sequenced commercially. Sequences were analysed via NCBI BLAST and deposited in GenBank for accession.

3.6 Preparation of Fungal Extracts

Each isolate was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of sterilised Potato Dextrose Broth (PDB). Flasks were incubated at 28 °C in a rotary shaker at 150 rpm for 21 days. After incubation, the fungal biomass was separated from the broth via filtration through sterile muslin cloth.

The filtrate was extracted thrice with equal volumes of ethyl acetate using a separatory funnel. Combined organic layers were dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure using a rotary evaporator. Crude extracts were weighed and reconstituted in DMSO to a final concentration of 10 mg/mL for further use.

3.7 Antimicrobial Screening

3.7.1 Test Organisms

A panel of bacterial and fungal pathogens was used:

- Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*
- Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*
- Fungal pathogen: *Candida albicans*
- All strains were obtained from MTCC (Microbial Type Culture Collection), Chandigarh, and maintained on Nutrient Agar (bacteria) and Sabouraud Dextrose Agar (fungi).

3.7.2 Agar Well Diffusion Assay

Muller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates were swabbed with microbial inocula adjusted to 0.5 McFarland standard (~1×10^8 CFU/mL). Wells (6 mm diameter) were punched and filled with 100 µL of fungal extract (10 mg/mL). DMSO served as the negative control, and ciprofloxacin (10 µg/mL) and fluconazole (25 µg/mL) as positive controls.

Plates were incubated at 37 °C for bacteria (24 h) and 30 °C for fungi (48 h). Zones of inhibition were measured in millimetres. Each assay was performed in triplicate.

3.8 Determination of Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method in 96-well microplates as per CLSI (2012) guidelines. Serial dilutions of crude extracts (ranging from 10 to 0.078 mg/mL) were prepared in MHB or SDB, inoculated with 100 µL of microbial culture, and incubated as appropriate. Resazurin dye (0.01%) was added to detect viability. The lowest concentration showing no colour change was recorded as the MIC.

3.9 Preliminary Phytochemical Analysis

Phytochemical screening of active extracts was conducted to detect key classes of secondary metabolites:

- **Alkaloids:** Wagner's reagent test
- **Flavonoids:** Alkaline reagent test
- **Tannins:** Ferric chloride test
- **Saponins:** Froth formation test
- **Terpenoids:** Salkowski test

Results were recorded as positive or negative based on colour change or precipitate formation.

3.10 TLC and Metabolite Profiling

TLC was conducted using silica gel plates and solvent systems including chloroform: methanol (9:1 and 8:2). Plates were visualised under UV light at 254 and 365 nm and by spraying with vanillin-sulphuric acid reagent. Active fractions were further profiled using LC-MS for mass determination and ^{1}H-NMR for structural analysis. These methods aimed to correlate chemical fingerprints with antimicrobial activity.

3.11 Statistical analysis: All results were expressed as mean ± standard deviation (SD). ANOVA was performed using SPSS (v25.0) to determine significance between mean inhibition zones of different fungal extracts. A p-value < 0.05 was considered statistically significant.

4. Results and Findings

The experimental study aimed at evaluating the antimicrobial potential of endophytic fungi isolated from

Ficus pumila resulted in multiple findings across several stages: isolation and characterisation of endophytes, crude extract preparation, antimicrobial screening, and secondary metabolite profiling. The following subsections present the detailed outcomes of each phase.

4.1 Fungal Isolation and Colonisation Frequency

From a total of 60 surface-sterilised tissue segments (20 each from roots, stems, and leaves), 26 morphologically distinct endophytic fungal isolates were recovered. The colonisation frequency (CF%) was highest in root tissues (65%), followed by stems (50%) and leaves (40%). These values are consistent with previous studies suggesting roots as a rich niche for fungal symbionts due to prolonged contact with soil microflora.

Table 1: Colonisation Frequency of Fungal Endophytes from Different Tissues of *Ficus pumila*

Tissue Type	Number of Segments	Colonised Segments	Colonisation Frequency (%)
Roots	20	13	65
Stems	20	10	50
Leaves	20	8	40
Total	60	31	51.6

All isolates were designated as FPEF1 to FPEF26 and maintained on PDA slants for further testing.

4.2 Morphological and Molecular Identification

Based on colony characteristics, pigmentation, spore morphology, and growth rate, the isolates were tentatively grouped under genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Colletotrichum*. Ten bioactive isolates showing significant antimicrobial activity were subjected to ITS sequencing. The sequences were compared against GenBank data using BLAST, and identities were confirmed with ≥98% similarity.

Table 2: Molecular Identification of Selected Fungal Endophytes

Isolate Code	Identified Species	Similarity (%)	GenBank Accession
FPEF2	*Penicillium citrinum*	99.3%	MN567801
FPEF5	*Fusarium oxysporum*	98.9%	MN567802
FPEF8	*Aspergillus flavus*	99.0%	MN567803
FPEF11	*Colletotrichum gloeosporioides*	98.8%	MN567804
FPEF14	*Trichoderma asperellum*	99.5%	MN567805

4.3 Antimicrobial Activity: Zone of Inhibition

All 10 selected isolates were tested against five microbial strains. Seven isolates exhibited measurable zones of inhibition, with FPEF2 and FPEF8 showing the most pronounced activity against *S. aureus* and *E. coli*. Extracts from FPEF14 (*T. asperellum*) demonstrated moderate activity against *Candida albicans*.

Table 3: Zone of Inhibition (mm) of Ethyl Acetate Extracts

Isolate	*S. aureus*	*E. coli*	*K. pneumoniae*	*B. subtilis*	*C. albicans*
FPEF2	21 ± 0.6	18 ± 0.8	16 ± 0.7	19 ± 1.0	14 ± 0.6
FPEF5	18 ± 1.1	14 ± 0.5	15 ± 0.6	17 ± 0.9	13 ± 0.4
FPEF8	22 ± 0.7	20 ± 0.9	18 ± 0.8	20 ± 0.8	16 ± 0.5
FPEF11	16 ± 0.5	13 ± 0.4	14 ± 0.5	15 ± 0.5	12 ± 0.3
FPEF14	14 ± 0.5	12 ± 0.3	13 ± 0.4	13 ± 0.4	17 ± 0.6
Control (Ciprofloxacin/Fluconazole)	26 ± 0.4	24 ± 0.6	25 ± 0.5	23 ± 0.5	22 ± 0.4

4.4 Minimum Inhibitory Concentration (MIC)

MICs for four most active isolates were determined. FPEF8 showed the lowest MIC values, confirming its high potency.

Table 4: MIC Values (mg/mL) of Active Extracts

Isolate	*S. aureus*	*E. coli*	*K. pneumoniae*	*B. subtilis*	*C. albicans*
FPEF2	0.625	1.25	1.25	0.625	1.25
FPEF5	1.25	2.5	2.5	1.25	1.25
FPEF8	0.312	0.625	0.625	0.625	0.625
FPEF14	1.25	1.25	1.25	1.25	0.625

4.5 Phytochemical Screening

Phytochemical analysis indicated a rich presence of secondary metabolites including flavonoids, alkaloids, and terpenoids in the bioactive extracts.

Table 5: Phytochemical Composition of Bioactive Extracts

Compound	FPEF2	FPEF5	FPEF8	FPEF14
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	-	+	+
Tannins	+	+	+	-
Saponins	-	-	+	+

(+ indicates presence; – indicates absence)

4.6 TLC and Spectral Analysis

TLC analysis of FPEF8 extract showed five distinct bands under UV light, suggesting chemical diversity. LC-MS analysis revealed mass ion peaks at m/z 261, 287, and 314, indicating possible flavonoid and alkaloid derivatives. Preliminary ^{1}H-NMR spectra exhibited aromatic protons (δ

7.2–7.6 ppm) and hydroxyl group signals, suggesting phenolic structures.
These findings indicate the presence of potentially novel bioactive compounds and justify further chromatographic purification and structural elucidation.

5. Discussion

The present investigation successfully establishes that endophytic fungi isolated from *Ficus pumila* possess considerable antimicrobial potential, providing promising leads for the discovery of novel bioactive compounds. The study not only reaffirms the antimicrobial biosynthetic capacity of endophytes but also supports the idea that medicinal plants and their microbiota may work in symbiosis to produce therapeutic effects. The results underscore the importance of exploring underutilised plants and their internal fungal communities in the search for next-generation antimicrobial agents.

5.1 Endophytic Diversity and Tissue Specificity

The recovery of 26 distinct endophytic fungal isolates from *Ficus pumila* confirms that the plant hosts a rich and diverse endophytic community. Consistent with previous research, root tissues showed the highest colonisation frequency, which may be attributed to their direct and prolonged contact with soil and microbial-rich rhizospheres (Arnold *et al*., 2000; Verma *et al*., 2009) [3, 29]. The colonisation frequency in stems and leaves, though relatively lower, still highlights the ability of these tissues to sustain microbial colonisers that remain asymptomatic.
Genera such as *Penicillium*, *Aspergillus*, *Fusarium*, *Colletotrichum*, and *Trichoderma* were predominant among the isolates. These genera are widely recognised for producing a broad spectrum of secondary metabolites, including antimicrobials, mycotoxins, and enzymes (Strobel & Daisy, 2003; Nicoletti & Fiorentino, 2015) [26, 17]. Their prevalence in *Ficus pumila* suggests that the plant serves as a favourable host for endophytic colonisation, especially by metabolically versatile fungi.

5.2 Antimicrobial Activity and Spectrum

The most striking result of the study was the broad-spectrum antimicrobial activity displayed by certain isolates, notably FPEF2 (*Penicillium citrinum*) and FPEF8 (*Aspergillus flavus*). These isolates showed inhibition against both Gram-positive and Gram-negative bacterial strains as well as the fungal pathogen *Candida albicans*. This suggests that the endophytes may produce chemically diverse compounds targeting multiple pathways, such as cell wall synthesis, protein biosynthesis, or membrane disruption.
The strong inhibition zones and low MIC values observed with FPEF8 highlight its potential as a high-priority candidate for bioassay-guided fractionation and metabolite purification. The MIC value of 0.312 mg/mL against *S. aureus* falls within the potent range defined for natural antimicrobial agents (Rios & Recio, 2005) [21]. These results compare favourably with existing literature where fungal endophytes isolated from plants such as *Azadirachta indica* and *Ocimum sanctum* have shown MIC values ranging between 0.5–1.25 mg/mL (Kharwar *et al*., 2011; Polpass *et al*., 2010) [12, 20].
Additionally, the activity against *Klebsiella pneumoniae*, a known multi-drug resistant organism, further strengthens the argument that endophyte-derived metabolites could offer unique structural scaffolds and modes of action distinct from current antibiotic classes (Ventola, 2015; Laxminarayan *et al*., 2013) [30, 15].

5.3 Phytochemical and Metabolite Insights

Phytochemical screening of crude extracts revealed the presence of alkaloids, flavonoids, terpenoids, and in some cases, saponins. These compound classes are commonly associated with antimicrobial effects in both plant and microbial sources (Cowan, 1999; Harborne, 1998) [7, 10]. Flavonoids are known to inhibit nucleic acid synthesis and disrupt membrane integrity, while terpenoids have been shown to interfere with microbial enzyme activity and membrane fluidity (Cushnie & Lamb, 2005) [8].
The LC-MS analysis provided valuable preliminary evidence regarding the molecular weights of bioactive constituents, and the observed m/z values suggest the presence of flavonoid or alkaloid-like structures. The ^{1}H-NMR data indicating aromatic proton signals further supports the likelihood of phenolic or aromatic compound classes, which are known for their broad antimicrobial activity (Zhang *et al*., 2006) [33].
Importantly, these results indicate that *Ficus pumila*-derived endophytes are metabolically active producers of secondary metabolites, potentially contributing to the plant's traditional use in infection-related ailments.

5.4 Novelty and Research Contribution

While the antimicrobial potential of endophytes from species like *Ficus religiosa* and *Ficus carica* has been documented, this is one of the first studies to systematically investigate the fungal endophytic community of *Ficus pumila*. The identification of metabolically active strains with significant antimicrobial activity adds a new dimension to the pharmacological understanding of this plant species and its microbiota.
Moreover, the application of a multifaceted methodological approach-combining classical microbiology with molecular and analytical chemistry-offers a template for future bioprospecting studies. The integration of phytochemical profiling and spectral analysis strengthens the scientific validity of the results and ensures that bioactive extracts are not only active but chemically traceable and characterisable.

5.5 Implications for Antimicrobial Resistance and Drug Discovery

The study's findings are particularly relevant in the current context of antimicrobial resistance (AMR), which has emerged as a formidable global challenge. As traditional antibiotics continue to lose efficacy, there is a critical need for new antimicrobial agents with novel targets or mechanisms of action (Davies & Davies, 2010; WHO, 2019) [9]. Natural products from microbial symbionts, especially fungal endophytes, offer such possibilities due to their evolutionary adaptations, diverse biosynthetic gene clusters, and unique metabolic pathways (Aly *et al*., 2010; Nicoletti & Fiorentino, 2015) [1, 17].
The potential application of these findings extends beyond human medicine. Some of the isolates also showed inhibition against *Candida albicans*, indicating possible use

in antifungal drug development. Additionally, the broad-spectrum activity of certain extracts may lend itself to applications in agriculture, particularly as biocontrol agents against phytopathogens-thereby contributing to sustainable farming practices (Schulz & Boyle, 2005) [22].

5.6 Limitations and Future Directions

While the study provides strong preliminary evidence of antimicrobial activity, it is not without limitations. Firstly, the crude extracts contain a complex mixture of compounds, and further purification is required to isolate and identify the specific bioactive principles. Secondly, *in vitro* antimicrobial testing, while indicative, does not fully replicate the complexity of *in vivo* conditions. Cytotoxicity and biocompatibility assays must also be conducted before any therapeutic use can be considered.

Future studies should focus on:

- Bioassay-guided fractionation and purification of active metabolites
- Whole-genome sequencing of promising isolates to identify biosynthetic gene clusters
- *In vivo* efficacy and toxicity studies in appropriate animal models
- Synergistic studies with existing antibiotics to evaluate combination potential

6. Conclusion

This study has demonstrated that endophytic fungi isolated from *Ficus pumila*, a traditionally important medicinal plant, harbour potent antimicrobial properties and biosynthetic capabilities. From a total of 26 isolates, several exhibited broad-spectrum activity against clinically relevant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Candida albicans*. Notably, isolates FPEF2 (*Penicillium citrinum*) and FPEF8 (*Aspergillus flavus*) showed substantial inhibition zones and low MIC values, highlighting their potential as lead candidates for further drug discovery research.

The phytochemical analysis indicated the presence of several known classes of bioactive secondary metabolites, including alkaloids, flavonoids, and terpenoids, which are likely contributors to the observed antimicrobial effects. In combination with the LC-MS and NMR spectral data, these findings offer strong preliminary evidence for the novelty and pharmaceutical relevance of metabolites produced by *Ficus pumila* endophytes.

This research contributes to the growing recognition of endophytic fungi as underexplored sources of novel antimicrobial compounds, particularly in the context of mounting global concern over antibiotic resistance. By focusing on a relatively unstudied plant species and applying a rigorous analytical framework, the study paves the way for future investigations that may lead to the development of new therapeutic agents.

Moreover, these findings hold promise not only for human health but also for sustainable agriculture, where such bioactive fungi could be deployed as natural biocontrol agents. Overall, *Ficus pumila*-derived endophytes offer a dual opportunity: one rooted in ancient traditional medicine and the other in cutting-edge microbial biotechnology.

Further purification of individual compounds, *in vivo* efficacy testing, and toxicity studies are essential next steps. However, this study offers a foundational step in recognising the antimicrobial potential of plant-associated fungal endophytes and their vital role in next-generation drug discovery pipelines.

7. References

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