Review

Endophytic Fungal Secondary Metabolites as Potential Drug Discovery Agents against Antimicrobial Resistance: A Systematic Review

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Posted Date: 27 November 2024

doi: 10.20944/preprints202411.2136.v1

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 doi:10.20944/preprints202411.2136.v1

Review

Endophytic Fungal Secondary Metabolites as Potential Drug Discovery Agents against Antimicrobial Resistance: A Systematic Review

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Abstract: Introduction: The rise of antimicrobial resistance has become a pressing global public health concern, necessitating the exploration of alternative antimicrobial agents. Endophytes have been recognized as a rich source of novel metabolites with potential antimicrobial properties.Aim: To comprehensively review the literature on secondary metabolites from endophytic fungi with antimicrobial activities against priority pathogens.Method: A systematic search was conducted in four electronic databases: PubMed, Scopus, Web of Science, and Google Scholar. Studies that reported the isolation and elucidation of secondary endophyte metabolites with inhibitory effects against clinically relevant bacteria and fungi were included. The Newcastle–Ottawa scale (NOS) was used to evaluate the methodological quality of the included studies.Results: Twenty-one studies met the inclusion criteria, and 77 specialised metabolites were characterised across the studies. These secondary metabolites exhibited broad-spectrum in vitro growth inhibition against priority pathogens, including bacteria such as *E. coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, and fungi such as *Candida albicans* and *Aspergillus* spp.Conclusion: This systematic review provides an overview of the current evidence on secondary endophyte metabolites with antibacterial and antifungal activities. The results highlight the potential of endophytes as a source of new antimicrobial agents.

Keywords: Endophytic fungi; secondary metabolites; antifungal; antibacterial; antimicrobial resistance

1. Introduction

Endophytic fungus inhabits healthy plant tissues without causing disease symptoms. They form beneficial symbiotic relationships with their hosts through mechanisms that remain largely elusive [1]. Although endophytes are thought to confer stress tolerance and nutrition, recent research indicates their deeper involvement in plant health and protection against pathogens [2]. Fungal secondary metabolites (SM) are important factors shaping these interactions [3]. SMs are mainly produced by filamentous fungi within the Pezizomycotina (Ascomycete) and various Basidiomycete classes, although some less expected fungi, such as Kluyveromyces lactis, also synthesise them. [4]. SMs are structurally diverse organic compounds produced via specialised metabolic pathways, such as photosynthesis, glycolysis and the Krebs cycle, and provide intermediates that lead to these natural products [5].

Although many bacteria and plants biosynthesize antimicrobial secondary metabolites, the metabolomes of endophytic fungi and their implications are only beginning to emerge. SMs from endophytic fungi have potential in pharmaceutical and agricultural applications because they

 doi:10.20944/preprints202411.2136.v1

possess antimicrobial properties [6]. Antimicrobial resistance poses a severe global threat, which is intensified by the shortage of new drug pipelines [7]. According to the WHO, drug-resistant infections already account for over 700, 000 annual deaths worldwide and are predicted to surpass cancer by 2050 if left unchecked [8]. Meanwhile, fungal infections steadily rise among immunocompromised patients [9], whereas available antifungal classes remain limited, and resistance spreads rapidly within medically important *Candida* and *Aspergillus* species [10]. Fungal SMs can be classified into four primary chemical categories: polyketide, terpenoids, compounds derived from shikimic acid, and non-ribosomal peptides [11], each offering unique chemical frameworks and bioactivities that hold immense potential as sources of novel therapeutics in the fight against antimicrobial resistance.

While primary studies have reported preliminary characterisation of endophytic fungal extracts and SMs, there is fragmentation within this nascent field that requires a consolidated analysis and perspectives to accelerate progress. Here, we review the development of SMs from endophytic fungi demonstrating antimicrobial activity, with a particular focus on activities against medically important drug-resistant bacteria and fungi that frequently cause serious infections.

2. Methodology

2.1. PRISMA Guidelines

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12].

2.2. Search Strategy

A comprehensive review of the literature was conducted to identify relevant articles published from January 1, 2010 to August 20, 2024 investigating secondary metabolites produced by endophytic fungi with antimicrobial activity. Databases searched included Scopus, Web of Science, PubMed, and Google Scholar. Search terms included combinations of "endophytic fungi", "secondary metabolites", "antimicrobial activity". Additional studies were sourced from the reference lists of relevant studies. The comprehensive search strategy is detailed in the supplementary table. The search results were imported into Rayyan software [13] for the removal of duplicates and screening.

2.3. Study Selection

Articles were initially screened based on titles and abstracts by two reviewers to determine their suitability for full-text review. Specific criteria were used to determine which articles would be included or excluded.

Inclusion criteria:

- Original research articles published in peer-reviewed journals from 2010-2023. This approach captured the current literature while providing sufficient data.
- Studies have isolated endophytic fungi from plants or environmental sources using standard procedures.
- Investigations using spectroscopic techniques like NMR, LC-MS to elucidate structures of fungal secondary metabolites.
- Reports determining the antibacterial and/or antifungal activity of metabolites/extracts using microdilution assays or disc diffusion tests.
- Studies stating minimum inhibitory concentration (MIC) values for bioactive compounds against target pathogens.

Exclusion criteria:

 doi:10.20944/preprints202411.2136.v1

- Review articles, book chapters, conference papers, and unpublished theses or dissertations. Note that these data do not represent primary data.
- Lack of details on fungal identification, compound structure elucidation, and antibacterial/antifungal evaluation methods.
- Investigations using endophytic actinomycetes or bacteria, with a specific focus on fungal secondary metabolites.
- Articles in languages other than English to maximise accessibility and analysis.

Two reviewers independently retrieved and assessed the full texts of potentially eligible studies. Any disagreements between the two reviewers at any stage of the screening process were resolved through consultation with a third reviewer.

2.4. Data Extraction

A standardised data extraction form was designed in an Excel spreadsheet and used to methodically gather relevant information from the selected studies. The following parameters were recorded for each publication:

- Authors and year of publication
- Fungal isolates, genus, and species (if identified); isolation source
- Isolated compounds: Name, chemical class, or structure
- Antimicrobial activity testing: target pathogens (bacterial/fungal strains),
- Activity against pathogens: Specified as demonstrated activity against the tested strains.

The two reviewers independently extracted the data. If agreement could not be achieved, a third reviewer was brought in to address and settle any discrepancies in the data extraction.

2.5. Quality Assessment

The methodological quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) [14]. The NOS assesses the studies across three main domains: selection, comparability, and outcome. Each domain is assigned a maximum number of stars, with a total of 9 possible stars. Specifically, the selection domain can receive up to 4 stars, the comparability domain up to 2 stars, and the outcome domain up to 3 stars. Studies with a total score of 6 or more stars were considered to be of high quality, while those with a score below 6 were deemed to be of lower quality. Two independent reviewers conducted the quality assessment, and any disagreements were resolved through discussion and consensus.

2.6. Data Synthesis and Analysis

A narrative synthesis was performed to summarise the findings of the included studies. Descriptive statistics included frequency distributions and percentages.

3. Results

3.1. Search Results

The initial database searches yielded a total of 1,914 records across Scopus, PubMed, and Web of Science. A manual search of the reference lists and Google Scholar identified an additional 26 articles, making a total of 1,940 articles (Figure 1).

After removing 426 duplicates, 1514 unique titles and abstracts were screened for eligibility.

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During the initial screening, 1218 articles were excluded because they did not meet the inclusion criteria, such as focusing on endophytic bacteria rather than fungi, or lacking characterisation of secondary metabolites and antimicrobial activities.

The remaining 296 potentially relevant studies were reviewed in full text. Of these, 275 were excluded for the following reasons:

- Not reporting original research (review articles, conference abstracts)
- Insufficient data on the isolation and structural characterisation of metabolites
- Lack of information about antimicrobial assessments

Ultimately, 21 studies were deemed eligible for inclusion in this review.

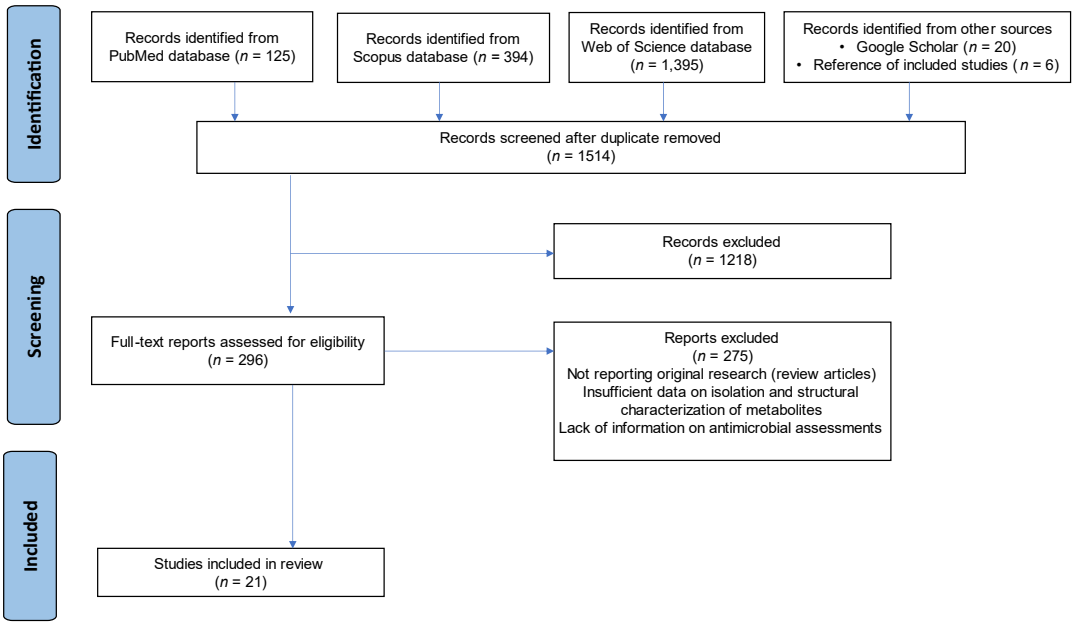

Figure 1. The flow diagram of the study selection process.

3.2. Sources of Endophytic Fungi

A wide range of plant species were studied as sources of endophytic fungi that produce secondary antimicrobial metabolites (**Table 1**). For instance, O. dillenii, an invasive plant in Sri Lanka, was found to harbour endophytic *Fusarium* spp. and *A. niger* [15]. The other species include Lagopsis supina (D. Zhang et al., 2023; Helicteres isora [17]; P. polyphylla var. yunnanensis [18]; M. azedarach L [19]; and Vinca plants [20]. The plant sources were roots of M. indica [21] and leaves of D. chinense [22], and leaves of Helianthus annuus [23]. However, non-plant sources were also studied, including seawater samples [24], and soft coral Sinularia spp. [25].

Table 1. Some plant species and sources.

Plant Species	Plant Source
Mangifera Indica	Roots
Catharanthus roseus	Healthy leaves
Rhizophora racemosa	Root
Melia azedarach L.	Stem bark
Distylium chinense	Leaves
Helianthus annuus	Leaves

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Eichhornia crassipes Linn	Leaves
Opontia dillenii	Cladodes and flowers
Rhizophora racemosa	Root
Olea europaea cv. Cobrançosa	Leaves
Polygonatum polyphyllum var. yunnanensis	Healthy rhizomes

3.3. Antimicrobial Activity Of Secondary Metabolites

The 21 studies identified 77 unique specialised metabolites that showed antimicrobial activity against bacteria and fungi. Twenty studies assessed the antibacterial effects against MDR bacterial pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *E. coli*, which are responsible for most nosocomial resistance worldwide. Sixteen studies assessed the antifungal effects of various medically important fungi, including *Candida albicans*, *Aspergillus fumigatus, and Aspergillus flavus* (**Table S2**).

3.4. Antimicrobial Activity Against Bacteria

3.4.1. Staphylococcus Aureus

Staphylococcus aureus is a ubiquitous commensal bacterium that can cause severe infections, such as pneumonia, bacteraemia, infective endocarditis, and toxic shock syndrome. Methicillin-resistant *S. aureus* (MRSA) is a leading cause of nosocomial infections because of its resistance to β-lactam antibiotics. Secondary metabolites from diverse fungal endophytes exhibit potency against *S. aureus*, including MRSA strains (**Table 2**). For instance, 1-H-indene 1-methanol acetate and azulene from *Curvularia eragrostidis* inhibited both standard and clinical MRSA strains [17]. Similarly, secondary metabolites, including altersolanol and fusaraichromenone produced by *Fusarium* spp., suppressed MRSA growth [26]. Violaceols from *Trichoderma polyalthiae* control methicillin-resistant *S. aureus* [27]. Sterols including 5α,8α-epidioxyergosta-6,22-dien-3β-ol and ergosta-7,22-dien-3β,5α,6β-triol, produced by *Pichia guilliermondii* exhibited antimicrobial activity against *S. aureus* [18]. Palitantin, fusarielin, and cytosporins isolated from *Pseudopestalotiopsis* spp., a mangrove endophyte, showed antimicrobial activity against *S. aureus* [28]. Metabolites including emodin, quesinol, and quesin from *Aspergillus* have been shown to exhibit activity against *S. aureus* [25]. Metabolites (nigerasperone C and asperpyrone A) of *Aspergillus niger* also restrict *S. aureus* growth [29]. Two secondary metabolites, 2-methoxy-6-methyl-1,4-benzoquinone and penicillic acid, isolated from *Aspergillus* and *Alternaria* species, demonstrated activity against *S. aureus* [16].

Table 2. Summary of bacterial pathogens, their targeted secondary metabolites, and the fungal sources.

Bacteria	Secondary metabolites	fungal source
Staphylococcus aureus	1-H-indene 1-methanol acetate, azulene	*Curvularia eragrostidis*
	Altersolanol, fusaraichromenone	*Fusarium* spp.
	Violaceols	*Trichoderma polyalthiae*
	5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosta-7,22-dien-3β,5α,6β-triol	*Pichia guilliermondii*
	Palitantin, fusarielin, cytosporins	*Pseudopestalotiopsis* spp.

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	Emodin, quesinol, quesin	*Aspergillus* spp.
	Nigerasperone C, asperpyrone A	*Aspergillus niger*
	2-methoxy-6-methyl-1,4-benzoquinone, penicillic acid	*Aspergillus, Alternaria*
Enterococcus spp. (*E. faecalis* and *E. faecium*)	Pterin-6 carboxylic acid, 1,2-benzenedicarboxylic acid	*Fusarium oxysporum*
	Rubrofusarin B, aspergillusol A	*Aspergillus niger*
Pseudomonas aeruginosa	Ergosta-5,7,22-trienol	*Pichia guilliermondii*
	Rubrofusarin B, fonsecin	*Aspergillus niger*
	1-H-indene 1-methanol acetate, azulene	*Curvularia eragrostidis*
	Emodin, quesinol, quesin	*Aspergillus* spp.
Escherichia coli	1-H-indene 1-methanol acetate	*Curvularia eragrostidis*
	Emodin, questinol, questin	*Aspergillus* spp.
	Palitantin, fusarielin, cytosporins	*Pseudopestalotiopsis* spp.
	Rubrofusarin B, aspergillusol A	*Aspergillus niger*
	2-methoxy-6-methyl-1,4-benzoquinone, penicillic acid	*Aspergillus, Alternaria*
Klebsiella pneumoniae	Ergosta-7,22-dien-3β,5α,6β-triol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol	*Pichia guilliermondii*
	9-Octadecenoic acid Z-, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester	*Aspergillus niger, Trichoderma lixii*
	Azulene, 1-H-indene 1-methanol acetate, N, N-diphenyl-2-nitro thio benzamide	*Curvularia eragrostidis*
Acinetobacter baumannii	9-Octadecenoic acid Z-, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester	*Aspergillus niger, Trichoderma lixii*
	Palitantin, cytosporins	*Pseudopestalotiopsis* spp.

3.4.2. Enterococcus spp.

Enterococcus species, such as *E. faecalis* and *E. faecium*, frequently cause infections in the urinary tract, bloodstream, and surgical sites. Given that intrinsic and acquired resistance compromise treatment options, new antimicrobials are urgently required. Secondary metabolites including pterin-6 carboxylic acid and 1,2-benzenedicarboxylic acid from *Fusarium oxysporum* inhibit vancomycin-resistant *Enterococcus* spp. [30]. Other secondary metabolites including rubrofusarin B and aspergillusol A from *Aspergillus niger* IMBC-NMTP0 also showed particular effectiveness against *E. faecalis* [29].

3.4.3. Pseudomonas Aeruginosa

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Pseudomonas aeruginosa is an opportunistic human pathogen that causes serious nosocomial infections in immunocompromised patients. Intrinsically resistant to many antibiotics poses a major challenge in treatment. Sterols, including ergosta-5,7,22-trienol, isolated from *Pichia guilliermondii* strongly inhibit *P. aeruginosa* growth [18]. Rubrofusarin B, fonsecin, and other *Aspergillus niger* metabolites similarly controlled *P. aeruginosa* [29]. The 1-H-indene 1-methanol acetate and azulene of *C. eragrostidis* are also particularly effective against *P. aeruginosa* [17]. Metabolites including emodin, quesinol, and quesin from *Aspergillus* showed activity against *P. aeruginosa* [25].

3.4.4. Escherichia Coli

Escherichia coli is the most prevalent cause of urinary tract and bloodstream infections worldwide. The mutations in outer membrane and porin, which contribute to intrinsic multidrug resistance, aggravate the crisis of emerging resistance. Many fungal endophyte extracts and secondary metabolites showed effectiveness against *E. coli*, including 1-H-indene 1 methanol acetate and sterols [17,18]. Similarly, secondary metabolites, including emodin, questinol, and questin from *Aspergillus*, were effective against *E. coli* [25]. Secondary metabolites including paluhyitantin, fusarielin, and cytosporins isolated from *Pseudopestalotiopsis* sp., a mangrove endophyte, showed antimicrobial activity against *E. coli* [28]. Additionally, secondary metabolites including rubrofusarin B and aspergillusol A from *Aspergillus niger* IMBC-NMTP0 showed particular effectiveness against *E. coli* [29]. Two secondary metabolites, 2-methoxy-6-methyl-1,4-benzoquinone and penicillic acid, isolated from *Aspergillus* and *Alternaria* species, demonstrated activity against *E. coli* [16].

3.4.5. Klebsiella Pneumoniae

Klebsiella pneumoniae is an important cause of serious nosocomial infections because of its resistance to many antibiotic classes. Several fungal endophyte secondary metabolites exert inhibitory effects against *K. pneumoniae*. For instance, sterols (including ergosta-7,22-dien-3β,5α,6β-triol, and 5α,8α-epidioxyergosta-6,22-dien-3β-ol) produced by *Pichia guilliermondii* exhibited antimicrobial activity against *K. pneumoniae* [18]. In another study, extracts containing ester secondary metabolites including 9-octadecenoic acid (Z)-, methyl ester and pentadecanoic acid, 14-methyl-, methyl ester of *Aspergillus niger* and *Trichoderma lixii* inhibited clinical strains of *K. pneumoniae* [21]. Similarly, secondary metabolites including azulene, 1-H-indene 1 methanol acetate and N, N-diphenyl-2-nitro thio benzamide from *C. eragrostidis*, also show particular effectiveness against *K. pneumoniae* [17].

3.4.6. Acinetobacter Baumannii

Acinetobacter baumannii has emerged as a significant drug-resistant nosocomial pathogen with limited treatment options. Compounds targeting *A. baumannii* are critical. The study by Abdelalatif et al. demonstrated that *A. niger* and *T. lixii* extracts containing secondary metabolites including 9-Octadecenoic acid (Z)-, methyl ester and pentadecanoic acid, 14-methyl-, methyl ester controlled multidrug-resistant *A. baumannii* growth [21]. Similarly, palitantin and cytosporins, which were secondary metabolites isolated from *Pseudopestalotiopsis* spp., a mangrove endophyte, showed antimicrobial activity against S. aureus [28].

3.5. Antimicrobial Activity Against Fungi

3.5.1. Candida Albicans

C. albicans is a common human pathogen that causes mucosal and systemic candidiasis. Several secondary metabolites exert inhibitory effects against this opportunistic yeast (**Table 3**). For instance, Violaceols from *Trichoderma polyalthiae* effectively restrict *C. albicans* growth [27]. Sterols including 5α,8α-epidioxyergosta-6,22-dien-3β-ol and ergosta-5,7,22-trienol isolated from *Pichia guilliermondii* also exhibited fungistatic properties against *C. albicans* [18]. Azulene and N, N-diphenyl-2-nitro thio benzamide produced by *Curvularia eragrostidis* exhibit antifungal activity against *C. albicans* [17].

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Palitantin and cytosporins isolated from *Pseudopestalotiopsis* spp., a mangrove endophyte, showed antimicrobial activity against *C. albicans* [28]. Secondary metabolites, including alternsolanol and fusaraichromenone produced by *Fusarium* spp. also suppress *C. albicans* growth [23]. Similarly, secondary metabolites, including Aspergillusol A and 2-(hydroxyimino)-3-(4-hydroxyphenyl) propanoic acid of *Aspergillus niger*, restrict *C. albicans* growth [29]. Other secondary metabolites including emodin, questinol, and questin, from *Aspergillus* spp. exhibited activity against *C. albicans* [25].

Table 3. Summary of fungal pathogens, their targeted secondary metabolites, and the fungal sources.

Fungi	Secondary metabolites	Fungi sources
Candida albicans	Violaceols	*Trichoderma polyalthiae*
	5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosta-5,7,22-trienol	*Pichia guilliermondii*
	Azulene, N, N-diphenyl-2-nitro thio benzamide	*Curvularia eragrostidis*
	Palitantin, cytosporins	*Pseudopestalotiopsis* spp.
	Alternsolanol, fusaraichromenone	*Fusarium* spp.
	Aspergillusol A, 2-(hydroxyimino)-3-(4-hydroxyphenyl) propanoic acid	*Aspergillus niger*
	Emodin, questinol, quesin	*Aspergillus* spp.
Trichophyton marneffei and *Microsporum gypseum*	2-phenylacetic acid, Z-methyl 4-(isobutyryloxy) but-3-enoate, 5-pentyldihydrofuran-2(3H)-one	*Nigrospora* spp.
Aspergillus spp.	Cladosporin, 50-hydroxyasperentin	Endophytic fungi in Zygophyllum mandavillei
	Azulene, 1-H-indene 1 methanol acetate, N, N-diphenyl-2-nitro thio benzamide	*Curvularia eragrostidis*
	Ergosta-7,22-dien-3β,5α,6β-triol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol	*Pichia guilliermondii*

3.5.2. Aspergillus spp.

A. fumigatus is an important cause of life-threatening infections in immunocompromised patients. Secondary metabolites, including Cladosporin and 50- hydroxyasperentin of endophytic fungi residing in Zygophyllum mandavillei inhibited the mycelial growth of *A. flavus* [31]. Azulene, 1-H-indene 1 methanol acetate and N, N-diphenyl-2-nitro thio benzamide from *C. eragrostidis* are also particularly effective against *A. fumigatus* [17]. Moreover, sterols (including 5α,8α-epidioxyergosta-6,22-dien-3β-ol and Ergosta-7,22-dien-3β,5α,6β-triol) produced by *Pichia guilliermondii*, exhibited antimicrobial activity against *A. niger* [18].

3.5.3. Dermatophyte

Several secondary metabolites suppress the causative agents of dermatophytosis (ringworm), which mainly affect skin, hair, and nails. For instance, 2-phenylacetic acid, (Z)-methyl 4-

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(isobutyryloxy) but-3-enoate, and 5-pentyldihydrofuran-2(3H)-one produced by the fungus *Nigrospora* spp. exhibit strong inhibitory effects against *Trichophyton marneffei* and *Microsporum gypseum* [23].

3.6. Risk of Bias Assessment

The 21 included studies underwent a methodological quality assessment. A substantial majority (18, or 86%) were determined to be of high quality, achieving ≥6 points on the NOS scale or demonstrating a low risk of bias across at least half of the assessed domains. Only three studies (14%) were rated as moderate quality, and none were found to be at high risk of bias (Table S2).

4. Discussion

This comprehensive review collated and analysed research progress regarding secondary antimicrobial metabolites isolated from endophytic fungi over the past few years. A wealth of studies reported the characterisation of structurally diverse bioactive compounds that display broad-spectrum inhibitory activities against an array of medically important bacterial and fungal pathogens.

One of the most remarkable aspects of the reviewed secondary metabolites is their ability to inhibit the growth of clinically relevant, drug-resistant bacteria. For instance, the potent antibacterial activities of compounds like pterin-6-carboxylic acid, 1,2-benzenedicarboxylic acid, and 1-docosene against *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* highlight their potential as alternative therapeutic options for the management of infections caused by these drug-resistant pathogens. Similarly, the antifungal activities of secondary metabolites, such as equisetin, altersolanol, and fusaraichromenone, against *Candida albicans* and *Cryptococcus neoformans* are particularly noteworthy. *Candida* species, including drug-resistant strains, are a leading cause of hospital-acquired infections [32], whereas *Cryptococcus neoformans* is a significant opportunistic pathogen in immunocompromised individuals [33]. The discovery of natural product-derived antifungal agents that can effectively inhibit the growth of these pathogens is a promising development in the ongoing efforts to combat the rise of antimicrobial resistance.

The diverse mechanisms of action underlying the antimicrobial activities of these secondary metabolites have been the subject of extensive investigation. Some compounds, such as the pterin-6-carboxylic acid and 1,2-benzenedicarboxylic acid isolated from *Fusarium oxysporum* and *Nigrospora sphaerica*, are believed to disrupt cell membrane integrity, leading to leakage of cellular contents and ultimately cell death [34]. Others, like the azulene compound from *Curvularia eragrostidis* HelS1, have been found to interfere with essential cellular processes, such as respiration and metabolism, thereby compromising the viability of the targeted pathogen [17]. The antibacterial and antifungal activities of secondary metabolites can also be attributed to their ability to inhibit specific enzymes or biochemical pathways crucial for microbial growth and survival. For instance, the chorismic acid analogue from *Aspergillus sydowii* SW9 is believed to disrupt the shikimate pathway, a critical metabolic route responsible for the synthesis of essential aromatic compounds in bacteria and fungi [35] Furthermore, some secondary metabolites can modulate the expression of virulence factors or quorum sensing systems in pathogens, thereby attenuating their ability to cause infection and disease [36] *Aspergillus niger* IMBC-NMTP0-derived compounds, such as asperpyrone A, nigerasperone A, and fonsecin, have been reported to exhibit this type of anti-virulence activity [29], highlighting the potential of these metabolites to serve as alternative antimicrobial strategies that do not directly target microbial growth.

The antimicrobial activities exhibited by the secondary metabolites have significant implications for their potential applications in various fields, including medicine, agriculture, and food preservation. In the agricultural sector, the antimicrobial properties of secondary metabolites can be exploited to develop eco-friendly biopesticide and biofungicides [37]. The ability of compounds like chaetoglobosin C and chaetoglobosin F to inhibit the growth of phytopathogenic fungi such as *Colletotrichum gloeosporioides*, *Gibberella saubinetii*, *Botrytis cinerea*, and *Alternaria solani* highlights their potential application in crop protection and disease management. Additionally, the antimicrobial

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activities of these secondary metabolites can be leveraged to preserve and protect food products [38]. The inhibition of foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli*, by compounds like 2-methoxy-6-methyl-1,4-benzoquinone and penicillic acid suggests their potential use as natural food preservatives, reducing the reliance on synthetic antimicrobial agents.

The vast diversity of microorganisms, including bacteria, fungi, and endophytic microbes, represents an immense resource for the identification of new antimicrobial lead compounds. However, despite the diversity covered, endophytic fungal metabolomes remain vastly understudied considering the estimated 1.5 million existing plant-fungal symbioses on Earth [39]. Detailed profiling using nuclear magnetic resonance and mass spectrometry is required to fully elucidate the modes of action and structure-activity relationships of the major active compound classes represented across the endophyte kingdom [40]. Further screening of extracts and identification of constituents active against a wider spectrum of priority pathogens, including emerging infectious strains, would help prioritise the most clinically translatable leads. Understanding taxonomic patterns associated with specialised metabolite repertoires may also aid such prioritisation towards prolific chemodiverse genera, such as *Aspergillus*, as evidenced by several studies in our review. Advances in culture-independent 'omic' methods can facilitate characterisation directly from environmental samples, thereby allowing access to much larger uncultured populations [41]. Metagenomic and metabolomic approaches offer paths to discover additional specialised metabolite biosynthetic clusters and chemical signatures without isolation [42].

However, the successful translation of these secondary metabolites into practical applications requires a multifaceted approach, including the optimisation of production and purification processes, evaluation of their safety and toxicity profiles, and development of effective formulations and delivery systems. We observed that most individual compounds characterised were only evaluated using standardised in vitro susceptibility assays. While these tests validate initial antibiotic properties, further efficacy assessments are needed using clinically relevant infection models that better mimic host-pathogen interactions before human use. Moreover, although individual compounds have potential as lead antimicrobials, synergistic interactions between multiple co-produced metabolites may underlie the high activity often observed in crude extracts[43]. Considerably more attention is required to unravel the combinatorial effects of these agents through biochemical and genetic studies. This approach has the potential to identify promising multicomponent combinations or 'cocktails' optimally inhibiting resistant pathogens. Collaborative efforts between researchers, industry, and regulatory authorities will be crucial in navigating the various challenges associated with the development and commercialisation of these natural product-based antimicrobial solutions.

5. Strengths and Limitations

This review highlights several promising antimicrobial secondary metabolites produced by endophytic fungi. However, there were also some limitations. Most studies relied on culture-based techniques, thus overlooking uncultured endophyte diversity. Additionally, pathogen screening panels were often narrow and did not sufficiently encompass the most pertinent multidrug-resistant strains. Metabolite yields from laboratory fermentation were generally low, indicating the need for improved production methods. Specific modes of action and structure-activity relationships have rarely been elucidated. Validation mostly used simplified in vitro assays rather than infection models. Very few investigations have progressed beyond initial screening to evaluate the safety, pharmacokinetics, and toxicology of clinical use.

6.Conclusions

This review underscored the remarkable antimicrobial potential of secondary metabolites derived from diverse endophytic fungi. The broad-spectrum activities exhibited by these compounds, which can effectively inhibit the growth of both Gram-positive and Gram-negative

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bacteria, as well as pathogenic fungi, highlight their immense value as a resource for the discovery and development of novel antimicrobial agents.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author contributions: Conceptualisation F.E.A-T., N.A-Q.M, and A.O; design: A.O., F.E.A-T, and N.A-Q.M.; formal analysis, A.O. F.E.A-T, and N.A-Q.M.; investigation, A.O. F.E.A-T., and N.A-Q.M.; data curation, A.O. F.E.A-T., and N.A-Q.M.; resources: F.E.A-T., N.A-Q.M, and A.O.; writing—original draft preparation: A.O., F.E.A-T., and N.A-Q.M.; writing—review and editing: A.O., F.E.A-T., and N.A-Q.M.; supervision: A.O.; project administration: A.O and F.E.A-T.; validation: A.O., F.E.A-T. All authors have read and agreed to the publication of the final version of the manuscript.

Funding: This review was not funded.

Data Availability Statement: All supporting data are presented in the manuscript and supplementary files.
Conflicts of Interest: The authors declare that they have no conflicts of interest

Ethics approval and consent to participate: Not applicable
Consent for publication: All authors have given their consent for the publication of this manuscript.
Acknowledgements: Not applicable

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