Review

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Bioactive Metabolites from Endophytic Fungi: Pharmaceutical Potential, Biomedical Applications, and Future Biotechnological Prospects

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Abstract

Endophytic fungi are microorganisms that reside asymptomatically within healthy plant tissues and establish complex symbiotic relationships with their host plants. In recent years, these microorganisms have gained increasing attention due to their remarkable ability to produce diverse secondary metabolites with significant biological and pharmacological activities. Numerous studies have demonstrated that endophytic fungi isolated from medicinal plants can synthesize bioactive compounds exhibiting antioxidant, antimicrobial, antiviral, anti-inflammatory, antidiabetic, and anticancer properties. Interestingly, some endophytes are capable of producing metabolites structurally similar or identical to those found in their host plants, including taxol, camptothecin, and various phenolic compounds. Compared with conventional plant extraction, endophytic fungi offer several advantages such as rapid growth, sustainable production, reduced environmental impact, and the potential for large-scale fermentation. These characteristics make endophytes promising alternative sources of natural products for pharmaceutical, cosmetic, agricultural, and industrial applications. This review summarizes the biological characteristics of endophytic fungi, methods for isolation and identification, and the major classes of bioactive compounds derived from fungal endophytes, with emphasis on their pharmacological significance and future biotechnological potential.

Keywords: endophytic fungi; bioactive compounds; secondary metabolites; medicinal plants; pharmaceutical biotechnology

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1. Introduction

Natural products have long played an essential role in drug discovery and pharmaceutical development (Tan & Zou, 2001; Strobel & Daisy, 2003). Many clinically important drugs, including antibiotics, anticancer agents, and immunosuppressive compounds, originate from natural sources such as plants, microorganisms, and marine organisms. Among these biological resources, medicinal plants have traditionally attracted considerable scientific interest because they contain a wide variety of bioactive metabolites with therapeutic potential (Kaul et al., 2012). However, direct extraction of valuable compounds from plants often faces several limitations, including low yield, slow plant growth, seasonal variation, habitat destruction, and high production costs.

In recent years, endophytic fungi have emerged as promising alternative sources of natural bioactive compounds. Endophytes are microorganisms that colonize internal plant tissues without causing visible disease symptoms in their host plants during at least part of their life cycle (Schulz & Boyle, 2005). These microorganisms establish mutualistic or symbiotic relationships with plants, where the host provides nutrients and shelter while the endophytes contribute to plant survival by producing protective metabolites and enhancing tolerance to environmental stress (Rodriguez et al., 2009).

Among endophytic microorganisms, fungal endophytes have received particular attention because of their remarkable metabolic diversity. Endophytic fungi are capable of synthesizing a broad spectrum of secondary metabolites, including alkaloids, terpenoids, flavonoids, quinones, steroids, peptides, phenolic compounds, and polyketides (Zhang et al., 2006). Many of these compounds exhibit important biological activities such as antioxidant, antimicrobial, antiviral, antidiabetic, anti-inflammatory, and anticancer effects (Kaul et al., 2012).

One of the most notable examples is the discovery of taxol-producing endophytic fungi isolated from species of Taxus. Paclitaxel (Taxol), an important anticancer drug originally isolated from yew trees, was later found to be produced by endophytic fungi associated with these plants (Stierle et al., 1993). Similar findings have been reported for other pharmacologically important compounds, including camptothecin, podophyllotoxin, and kojic acid (Puri et al., 2005; Kusari et al., 2012).

The increasing prevalence of antimicrobial resistance, chronic diseases, and cancer has intensified the search for novel therapeutic agents from natural resources. In this context, fungal endophytes represent an important and relatively underexplored reservoir of bioactive metabolites (Hyde et al., 2019). The ecological interaction between medicinal plants and endophytic fungi is summarized in Figure 1, highlighting fungal colonization within plant tissues and the mutual benefits associated with this symbiotic relationship.

Unlike previous reviews that primarily focus on the diversity of endophytic fungi or isolated classes of natural products, this review provides an integrated perspective on fungal-derived bioactive metabolites, their pharmaceutical and biomedical applications, and future biotechnological opportunities for sustainable metabolite production.

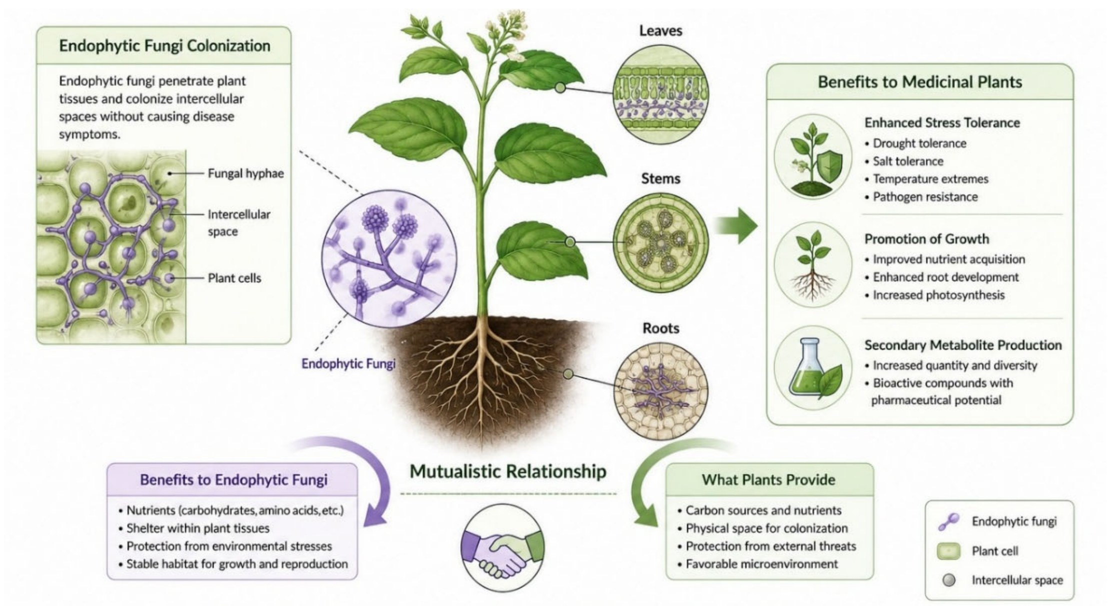

Figure 1. Ecological Relationship Between Medicinal Plants and Endophytic Fungi. Legend: Ecological relationship between medicinal plants and endophytic fungi. Endophytic fungi inhabit internal tissues of medicinal plants, such as leaves, stems, and roots, without inducing visible disease symptoms. This symbiotic interaction supports plant growth, stress adaptation, and secondary metabolite production, whereas medicinal plants offer nutrients, shelter, and a favorable microenvironment for fungal colonization. Adapted from concepts described by: Rodriguez et al. (2009), Schulz & Boyle (2005), Bacon & White (2000).

Importantly, this review emphasizes a systems-level conceptual framework that integrates ecological context, biosynthetic gene clusters, metabolomic outputs, and bioprocess scalability. This

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integrative perspective aims to move the field beyond descriptive cataloguing toward predictive and application-oriented bioprospecting of endophytic fungi.

2. Literature Search Strategy

Relevant literature was collected from electronic databases including PubMed, Scopus, Web of Science, and Google Scholar. The search was conducted using combinations of keywords such as "endophytic fungi", "bioactive metabolites", "secondary metabolites", "medicinal plants", "antioxidant", "antimicrobial", "anticancer", and "pharmaceutical applications".

Articles published in English and relevant to fungal endophytes and their bioactive compounds were prioritized. Original research articles, review papers, and book chapters published from 1991 to 2025 were considered for inclusion. Additional references were identified through manual screening of cited literature from relevant publications.

3. Biological Characteristics of Endophytic Fungi

Endophytic fungi are fungal microorganisms that inhabit internal tissues of healthy plants without inducing visible symptoms of disease (Petrini, 1991). They may colonize various plant organs, including roots, stems, leaves, flowers, seeds, and bark. These fungi are widely distributed in nature and have been identified in nearly all plant species examined to date (Rodriguez et al., 2009).

The relationship between endophytic fungi and host plants is generally considered mutualistic. Plants provide nutrients, carbon sources, and a protected ecological niche for fungal growth, whereas endophytes enhance plant survival through several mechanisms. These include the production of antimicrobial compounds, phytohormones, siderophores, and stress-protective metabolites (Arnold et al., 2003). Endophytes may also improve plant resistance against pathogens, herbivores, drought, salinity, and oxidative stress (Saikkonen et al., 1998).

Endophytic fungi belong predominantly to the phyla Ascomycota and Basidiomycota, although representatives of other fungal groups have also been reported. Their colonization strategies vary depending on fungal species, plant host, environmental conditions, and tissue specificity. The general workflow for isolation and preliminary morphological identification of endophytic fungi from medicinal plants is illustrated in **Figure 2**.

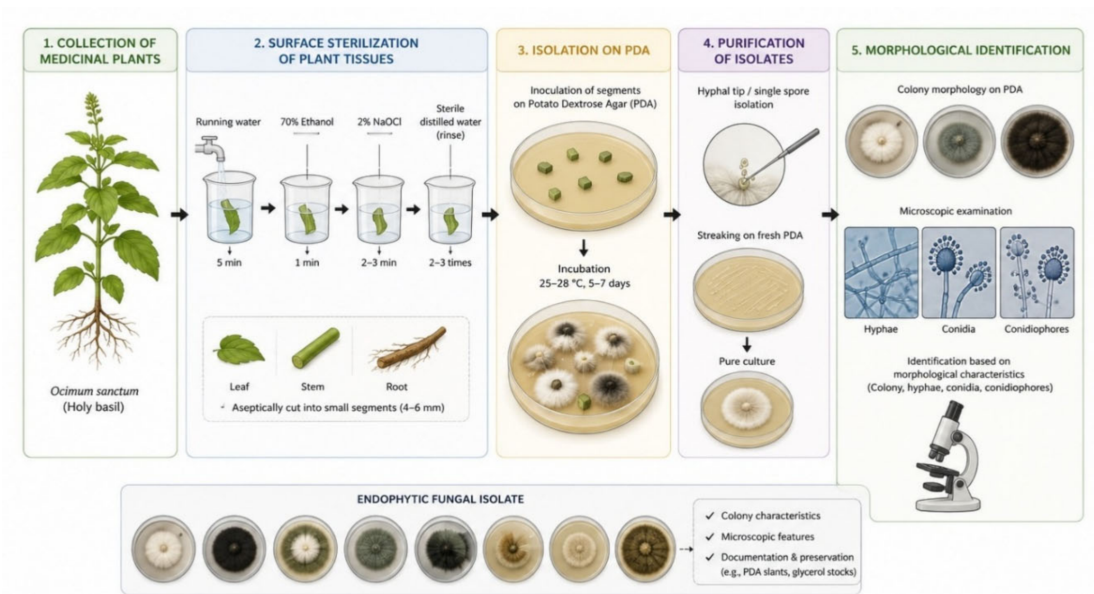

Figure 2. Isolation and Morphological Identification of Endophytic Fungi. Legend: Isolation and morphological characterization of endophytic fungi from medicinal plants. Surface-sterilized plant tissues are cultured on

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potato dextrose agar (PDA), followed by purification and microscopic examination of fungal isolates for preliminary identification. Adapted from methods described by Verma et al. (2009) and Hyde et al. (2019).

4. Isolation and Identification of Endophytic Fungi

The isolation of endophytic fungi begins with the selection of healthy and disease-free plant materials. Samples should ideally be processed within 24 hours after collection to maintain microbial viability and minimize contamination (Verma et al., 2009).

Surface sterilization is a critical step in the isolation procedure because it removes epiphytic microorganisms while preserving internal endophytes. Typically, plant samples are washed thoroughly under running water and treated sequentially with ethanol and sodium hypochlorite solutions, followed by rinsing with sterile distilled water (Schulz & Boyle, 2005).

After sterilization, plant tissues are cut into small segments using sterile instruments and placed on culture media such as potato dextrose agar (PDA) supplemented with antibiotics to inhibit bacterial growth. Plates are incubated at approximately 25 °C, and emerging fungal hyphae are subcultured repeatedly to obtain pure isolates. The workflow for the isolation and identification of endophytic fungi from medicinal plants is illustrated in **Figure 3**.

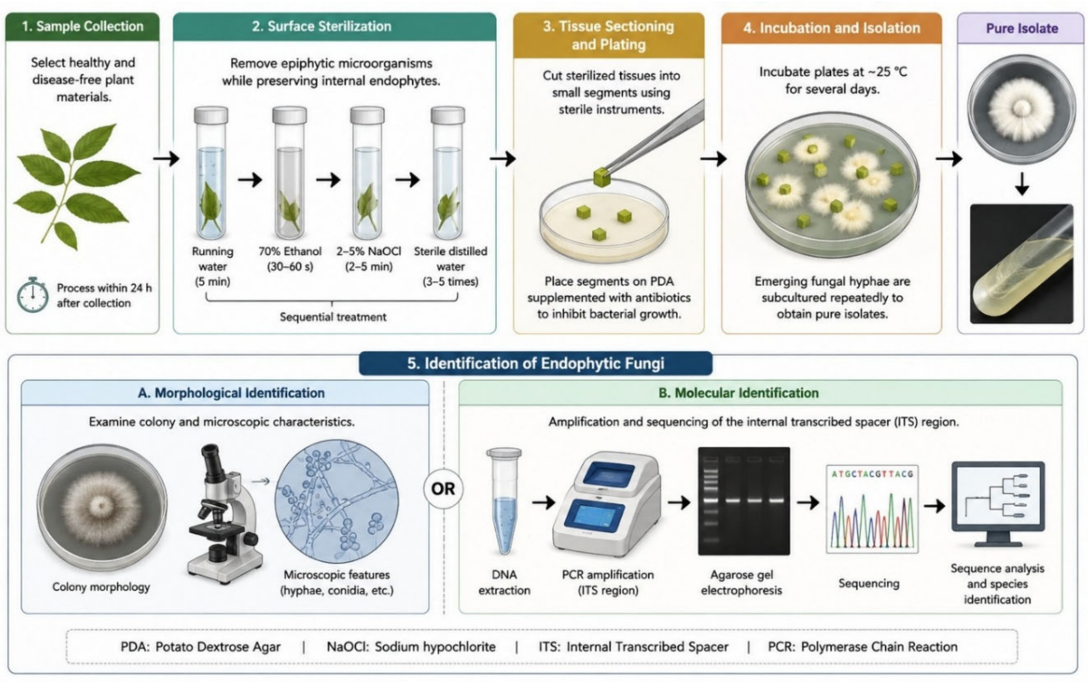

Figure 3. Isolation and Identification of Endophytic Fungi from Medicinal Plants. Legend: Workflow illustrating the isolation and identification of endophytic fungi from medicinal plants. Healthy plant tissues are collected and surface sterilized to eliminate epiphytic microorganisms, followed by aseptic sectioning and cultivation on potato dextrose agar (PDA). Emerging fungal colonies are purified through subculturing and subsequently characterized using morphological observation and molecular identification based on internal transcribed spacer (ITS) region amplification and sequencing. Adapted from methodologies described by Verma et al. (2009) and Schulz and Boyle (2005).

Identification of endophytic fungi can be performed using both morphological and molecular approaches. Molecular identification commonly involves amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA using polymerase chain reaction (PCR).

Recent advances in molecular identification techniques, including next-generation sequencing (NGS), metagenomics, and phylogenetic analysis, have significantly improved the detection and

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characterization of unculturable or rare endophytic fungal taxa, thereby expanding opportunities for novel metabolite discovery (Hyde et al., 2019; Kusari et al., 2012).

5. Bioactive Compounds from Endophytic Fungi

Endophytic fungi produce a wide variety of bioactive secondary metabolites with important pharmacological properties. The major classes of metabolites synthesized by these fungi and their associated biological activities are summarized in **Figure 4**.

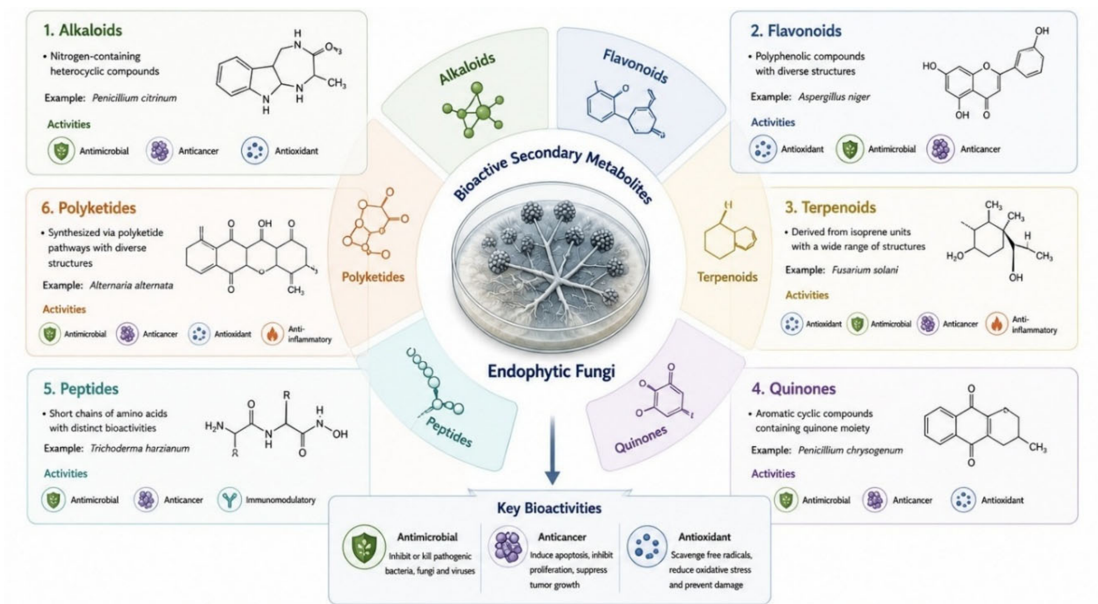

Figure 4. Bioactive Secondary Metabolites Produced by Endophytic Fungi. Legend: Bioactive secondary metabolites produced by endophytic fungi. Endophytic fungi synthesize diverse classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, quinones, peptides, and polyketides. These bioactive compounds possess important pharmacological properties, such as antioxidant, antimicrobial, and anticancer activities, supporting their potential applications in pharmaceutical and biomedical research. Adapted from Kaul et al. (2012), Kusari et al. (2012), and Zhang et al. (2006).

5.1. Antioxidant Compounds

Oxidative stress caused by reactive oxygen species (ROS) is associated with numerous pathological conditions, including cancer, cardiovascular disease, neurodegenerative disorders, diabetes, and aging. Consequently, antioxidants play an important role in preventing cellular damage and maintaining physiological balance (Cowan, 1999).

Many endophytic fungi have been reported to produce antioxidant compounds such as phenolics, flavonoids, quinones, and polysaccharides (Kaul et al., 2012). For example, Xylaria species isolated from Ginkgo biloba were found to produce phenolic metabolites with strong free radical scavenging activity.

5.2. Antimicrobial Compounds

Endophytic fungi produce a wide range of antimicrobial metabolites that protect both the fungi and their host plants from pathogenic microorganisms (Strobel & Daisy, 2003). These metabolites include alkaloids, terpenoids, peptides, quinones, and polyketides with antibacterial, antifungal, antiviral, and antiparasitic activities (Tan & Zou, 2001).

The growing problem of antimicrobial resistance has increased interest in fungal endophytes as alternative sources of novel antimicrobial agents (Hyde et al., 2019).

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5.3. Anticancer Compounds

Among the most significant discoveries involving endophytic fungi is their ability to produce anticancer compounds. Paclitaxel-producing endophytic fungi were first reported from Taxus species by Stierle et al. (1993).

Other anticancer metabolites produced by endophytic fungi include camptothecin, terrein, penicillenols, and mycoepoxydiene. These compounds exhibit cytotoxic activities against various cancer cell lines, including breast, lung, cervical, liver, and leukemia cells.

Representative anticancer metabolites produced by endophytic fungi and their associated biological activities are summarized in Figure 5. Table 1.

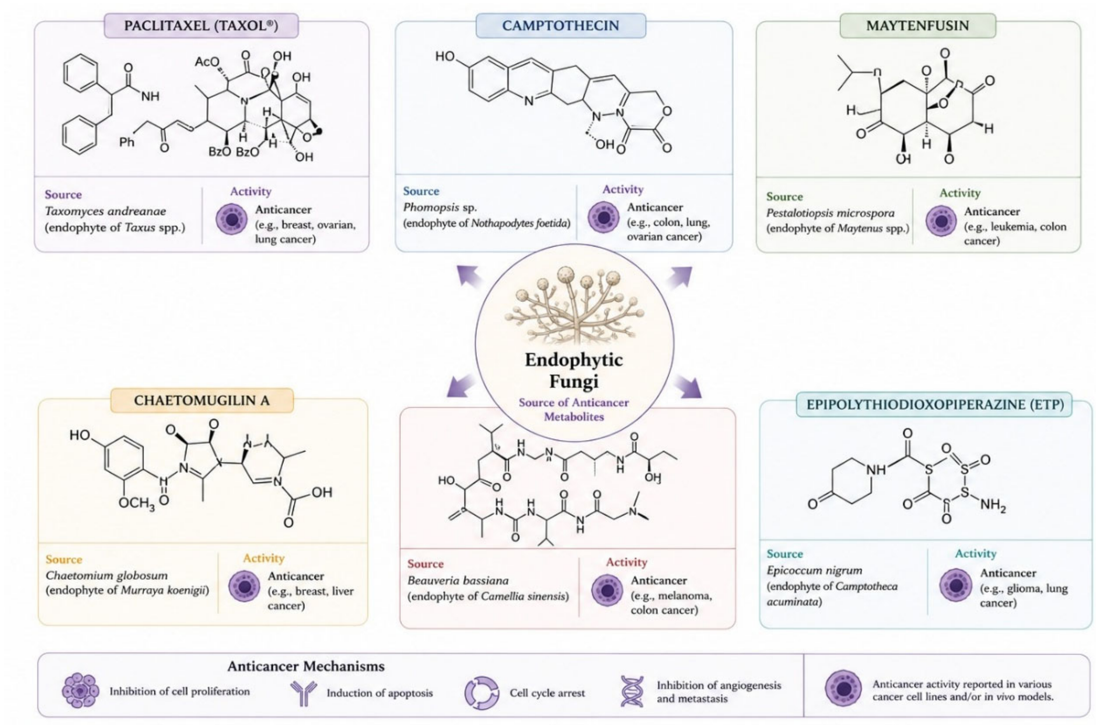

Figure 5. Anticancer Metabolites Produced by Endophytic Fungi. Legend: Representative anticancer metabolites produced by endophytic fungi, including paclitaxel, camptothecin, chaetomugilin A, and epipolythiodioxopiperazines (ETPs), along with related compounds. These metabolites exhibit diverse anticancer mechanisms, including inhibition of cell proliferation, induction of apoptosis, cell cycle arrest, and suppression of angiogenesis and metastasis. Adapted from Stierle et al. (1993), Puri et al. (2005), Kaul et al. (2012), and Kusari et al. (2012).

A summary of the major classes of bioactive metabolites produced by endophytic fungi and their associated biological activities is presented in **Table 1**.

Table 1. Major classes of bioactive metabolites produced by endophytic fungi and their representative biological activities.

Metabolite class	Representative compounds	Major biological activities	References
Alkaloids	Camptothecin, vinblastine-like compounds	Anticancer, antimicrobial	Puri et al. (2005); Kaul et al. (2012)
Terpenoids	Taxol (paclitaxel), terrein	Anticancer, anti-inflammatory	Stierle et al. (1993); Kusari et al. (2012)

Flavonoids	Flavones, flavonols	Antioxidant, anti-inflammatory	Kaul et al. (2012)
Quinones	Anthraquinones, naphthoquinones	Antimicrobial, anticancer	Zhang et al. (2006)
Peptides	Cyclic peptides, peptaibols	Antimicrobial, cytotoxic	Strobel & Daisy (2003)
Polyketides	Mycoepoxydiene, penicillenols	Anticancer, antimicrobial	Kusari et al. (2012)
Phenolic compounds	Phenolic acids, tannin-like compounds	Antioxidant, antimicrobial	Kaul et al. (2012)

6. Applications of Endophytic Fungi

Bioactive metabolites produced by endophytic fungi have broad applications in pharmaceutical, biomedical, agricultural, cosmetic, and industrial fields. The major application areas of these fungal metabolites are summarized in Figure 6.

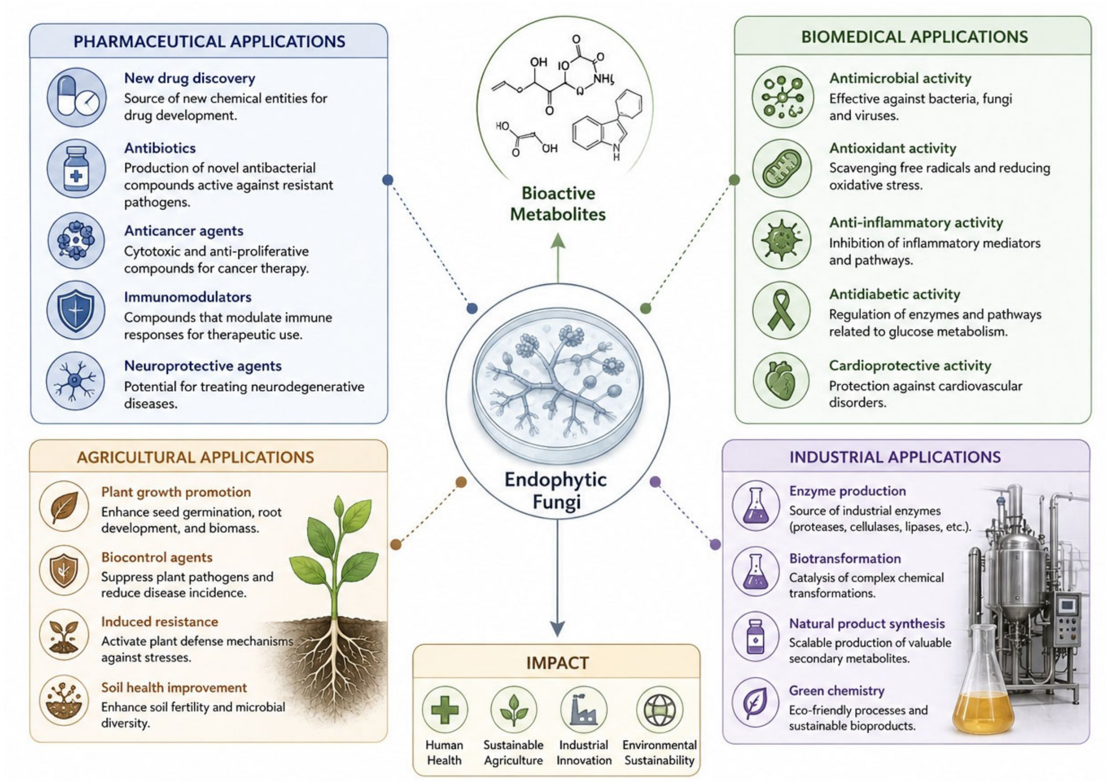

Figure 6. Pharmaceutical and Biomedical Applications. Legend: Pharmaceutical, biomedical, agricultural, and industrial applications of bioactive metabolites derived from endophytic fungi, including antibiotics, anticancer agents, antioxidants, cosmetic ingredients, and agricultural biocontrol compounds. Adapted from Hyde et al. (2019) and Nicoletti and Fiorentino (2015).

The bioactive metabolites produced by endophytic fungi have broad applications in multiple fields. In medicine, fungal metabolites may serve as antibiotics, anticancer agents, antioxidants, immunosuppressants, and anti-inflammatory drugs (Strobel & Daisy, 2003; Nicoletti & Fiorentino, 2015).

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In agriculture, endophytes can function as biological control agents that enhance plant growth and resistance to pathogens (Rodriguez et al., 2009; Hyde et al., 2019).

In the cosmetic industry, compounds such as kojic acid are widely used in skin-whitening and anti-aging products because of their antioxidant and tyrosinase-inhibitory activities (Kusari et al., 2012).

Furthermore, advances in biotechnology and fermentation technology have enabled large-scale cultivation of fungal endophytes for metabolite production. Compared with harvesting medicinal plants, microbial fermentation offers a more sustainable and environmentally friendly production system (Hyde et al., 2019). In addition to conventional fermentation systems, metabolic engineering and strain improvement strategies have been increasingly explored to enhance the yield and stability of fungal metabolite production for pharmaceutical and industrial applications.

7. Future Perspectives and Clinical Challenges

Although significant progress has been achieved in the study of endophytic fungi as sources of bioactive secondary metabolites, the field is still limited by several key biological and practical bottlenecks that affect their full application in pharmaceutical and industrial development. One of the main issues is the inconsistency of metabolite production under laboratory conditions. Many endophytic fungi show strong dependence on host origin and environmental conditions, which leads to variation in their metabolic profiles when they are cultured in different systems. This makes it difficult to reliably use laboratory screening as a direct indicator of their natural biosynthetic potential. In addition, a large number of endophytic fungi cannot be easily cultured, or they remain metabolically inactive under standard laboratory conditions, which means that much of their chemical diversity is still not accessible. Even in strains that can be cultured, many biosynthetic gene clusters are silent, suggesting that only a small portion of their true metabolic capacity is currently being expressed.

Another important limitation is the lack of a continuous workflow from fungal isolation to metabolite development. In many studies, each step is still done separately, from screening to compound identification, without proper integration into strain improvement or production optimization. Because of this gap, many interesting metabolites stop at the early research stage and are never developed further.

Furthermore, although omics technologies have developed rapidly, there is still a clear gap between what is predicted from genome mining and what is actually produced in the laboratory. At the moment, there is no reliable framework that can fully connect fungal ecology, host specificity, gene cluster activation, and real metabolite production under scalable conditions. This means that many studies still move separately through discovery, validation, and application, rather than being connected in a continuous process. As a result, computational predictions of biosynthetic potential do not always match the compounds that are finally confirmed in chemical or biological tests. In addition, there is still no proper system that integrates fungal taxonomy, ecological information, gene clusters, metabolomic data, and production strategies in a way that allows researchers to predict which strains are truly valuable. Without this integration, research in this area remains mostly descriptive rather than predictive.

Future research should focus more on combining multi-omics approaches such as genomics, transcriptomics, metabolomics, and epigenomics with data-driven tools to help identify promising strains and activate silent gene clusters. At the same time, synthetic biology and heterologous expression systems should be further improved to overcome the limitations of unculturable or low-yield strains. Research should also move beyond studying single fungal isolates and start to consider endophytic fungi as part of a larger plant-associated microbial community, since their behavior in nature is likely influenced by interactions with other microorganisms and the host plant. Finally, there is a clear need for standardized methods in isolation, screening, and fermentation so that results can be compared more reliably across different studies and more easily translated into real applications.

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8. Conclusions

Endophytic fungi represent a valuable and sustainable source of biologically active secondary metabolites with diverse pharmacological properties. These microorganisms produce a wide variety of compounds exhibiting antioxidant, antimicrobial, anticancer, and other therapeutic activities. Their ability to synthesize metabolites similar to those of medicinal plants highlights their importance in natural product research and drug discovery.

With continued advances in molecular biology, biotechnology, and metabolite screening techniques, endophytic fungi are expected to play an increasingly important role in the development of new pharmaceuticals, agricultural products, and industrial applications. Further exploration of fungal biodiversity, particularly in medicinal plants from tropical regions, may lead to the discovery of novel compounds with significant biomedical potential.

Overall, endophytic fungi represent an underexplored yet highly promising reservoir of natural products for future pharmaceutical innovation. Continued interdisciplinary research combining mycology, biotechnology, medicinal chemistry, and systems biology will be essential for translating fungal metabolites into clinically and industrially relevant products.

Author Contributions: Monthon Lertcanawanichakul: Project administration, Supervision, Conceptualization, Literature review, Writing – original draft, Visualization, Manuscript preparation, Correspondence, Final approval of the manuscript. Attarat Pattanawongsa: Literature review, Writing – review & editing, Data curation, Validation, Final approval of the manuscript. Sueptrakool Wisetsombat: Critical revision, Writing – review & editing, Final approval of the manuscript.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: During the preparation of this manuscript, graphical layouts and schematic illustrations were developed using AI-assisted image generation tools for conceptual scientific visualization. All scientific content, figure selection, labeling, interpretation, and manuscript text were reviewed and approved by the authors. No AI tools were used for data fabrication, scientific analysis, or reference generation.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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