

Bioactive Secondary Metabolites from Endophytic Fungi: Advances in Isolation, Structural Characterization and Pharmacological Activities

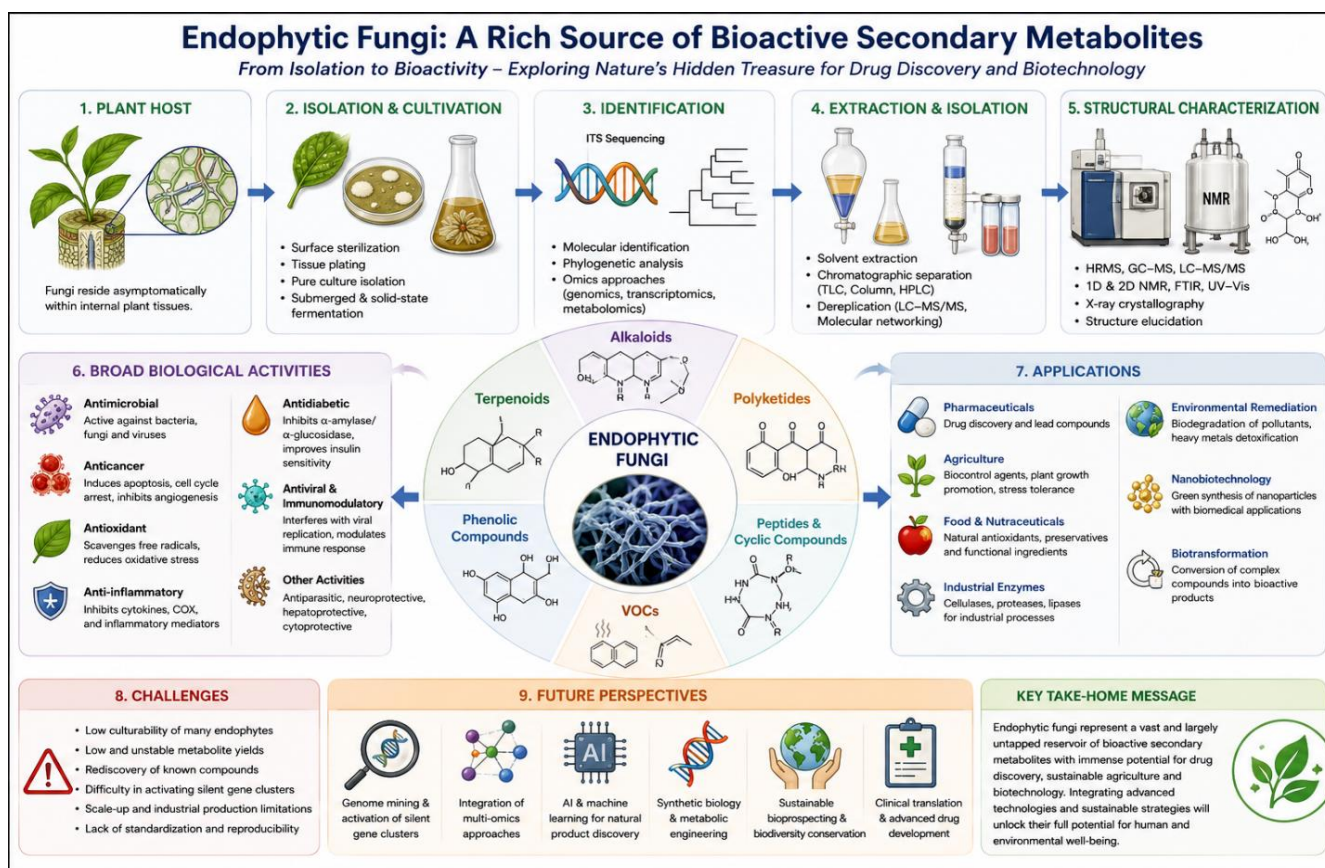
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GRAPHICAL ABSTRACT



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Abstract: Endophytic fungi are microbes living asymptotically within the internal tissues of plants-that have attracted much attention recently as promising sources of pharmacologically and biotechnologically relevant, structurally diverse, secondary metabolites, such as alkaloids, terpenoids, polyketides, phenolics, peptides and volatile organic compounds, which have possessed interesting antimicrobial, anti-cancer, antioxidant, anti-inflammatory and anti-diabetic activities. The novelty of the compounds produced, even some structures being analogues to plant-derived compounds and the discovery potential to be interesting alternative for natural product discovery, has led the endophytic fungi as a valuable source for natural products discovery. The progress in cultivation techniques, isolation and molecular identification (e.g., ITS sequencing) and omics technologies (genomics, transcriptomics and

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metabolomics) in the past decade made the metabolite discovery possible and characterization easier. Endophytic fungi. Genome mining identified a number of silent biosynthetic gene clusters which suggest a large number of un-explored bioactive compounds produced by fungi. Additionally, as well as having a pharmaceutical perspective, endophytic fungi have properties such as plant growth promotion, environmental stress resistance and effects on the plant and ecosystem within agricultural and environmental contexts. In spite of these advances, issues such as low yield of metabolites, challenging culturing of specific fungal strains, difficulty in de-replication of known metabolites and scale up remain significant obstacles to industrial exploitation. This review summarizes recent discoveries in the isolation, structural characterization and pharmacological potential of secondary metabolites produced by endophytic fungi.

1. Introduction

Endophytic fungi are a group of micro-organisms which dwell within inner plant tissues without visible disease signs during their life cycle or parts of it. Endophytic fungi, have become a prominent element of plant associated micro-biome, found everywhere, in aquatic and terrestrial environments (Selvakumar *et al.*, 2018; Du *et al.*, 2026; Compant *et al.*, 2021). Endophytes are the micro-organisms that colonize inner plant tissues asymptotically in an obligate or facultative mutualistic association with their hosts plants (Bogas *et al.*, 2024). True endophytes are reported to live within the plants without disease symptoms in nature, differentiating them from latent pathogens (Du *et al.*, 2026).

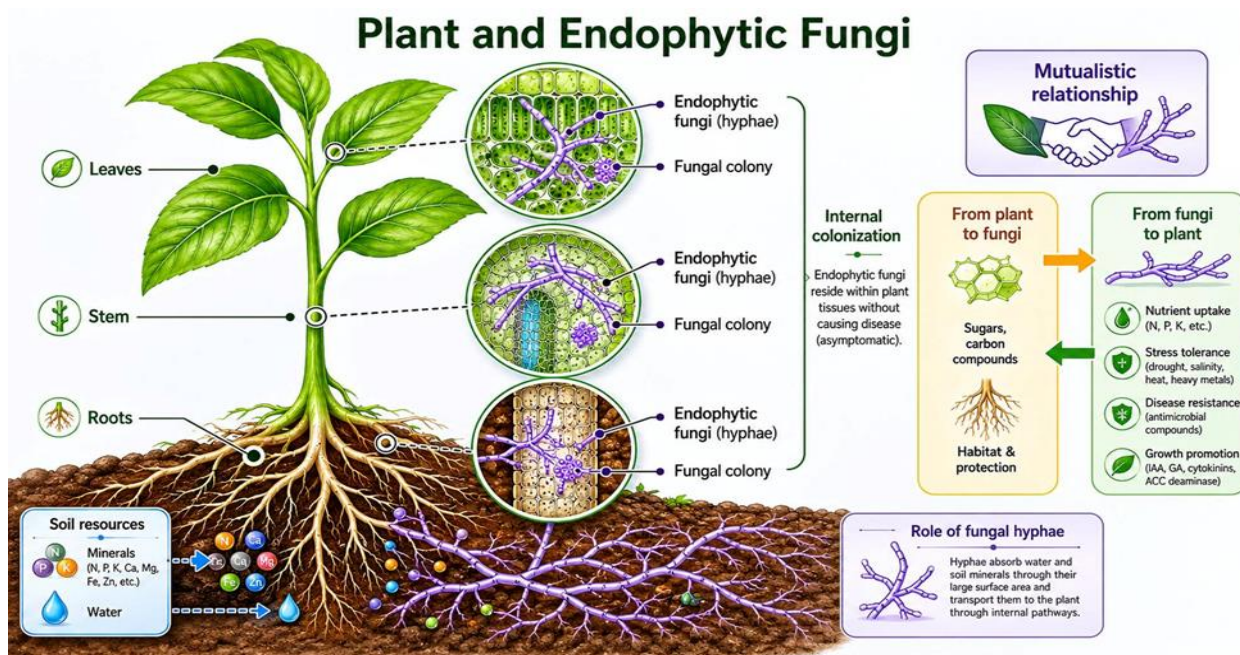


Figure 1: Plant and endophytic fungi

1.1 Historical Background

Although the notion of endophytic fungi originated from early microscopic examinations made in the 19th century, it has gained significant attention from the late 20th century after the isolation of biologically active metabolites produced by fungi (Raimi and Adeleke, 2021). A turning point in research, which has been an inspiration to develop fungal endophytes as an alternative source of plant derived drugs, was the isolation of Taxol producing endophytic fungi from *Taxus* spp.

(Selvakumar *et al.*, 2018; Raimi and Adeleke, 2021; Alla *et al.*, 2026). After this, research has accelerated due to their ecological and pharmacological significance (Du *et al.*, 2026).

1.2 Diversity and Ecological Distribution

Endophytic fungi have been isolated from all plant phyla i.e. Bryophytes, pteridophytes, gymnosperms and angiosperms, indicating they are widespread organisms (Zheng *et al.*, 2016; Du *et al.*, 2026). The majority of the endophytic fungi identified belong to phyla Ascomycota and Basidiomycota, with some represented by other phyla (Selvakumar *et al.*, 2018). Endophyte diversity is affected by a range of factors including; host plant species, type of tissue examined, environmental condition, geographical origin and climatic condition (Zheng *et al.*, 2016). Higher fungal diversity is usually observed in tropical regions due to more appropriate humidity and temperature, this leads to better conditions for fungal growth (Bogas *et al.*, 2024). Also, it has been noted that the tissue of the host plant plays an important role in the composition of microbes, indicating ecological adaptation and co-dependence between plant and microbe (Du *et al.*, 2026).

The introduction of modern molecular techniques like the ITS sequencing, metagenomics, next-generation sequencing and etc has showed the actual high diversity of the endophytic fungi, unlike the culture methods that underestimated the diversity of the fungi present in plants (Selvakumar *et al.*, 2018). These researches show that endophytic fungi represent an inexhaustible reservoir for biodiversity and natural products (Du *et al.*, 2026; White *et al.*, 2019).

1.3 Significance in Natural Products Chemistry

The natural products continue to be an extremely significant source of drugs, contributing to most approved drugs especially in oncology and infectious diseases (Newman and Cragg, 2020) since they often possess appropriate structural characteristics for acting as potential lead compounds for medical chemistry due to their high degree of chemical specificity (Atanasov *et al.*, 2021). Natural products can provide diversity to feed the discovery pipeline, where synthesis, although improving fast, has no chance of competing (Davison and Brimble, 2019; Atanasov *et al.*, 2021).

1.4 Endophytes as Producers of Secondary Metabolites

Endophytes are being explored for the rich diversity of secondary metabolites produced by them (e.g. Polyketides, alkaloids, terpenoids, peptides, phenols, etc.) (Patil *et al.*, 2016; Singh & Kumar, 2023; Aourabi *et al.*, 2021; Diass *et al.*, 2021; Singh *et al.*, 2021) that often demonstrate broad-spectrum activities such as antibacterial, anticancer, antioxidant and anti-inflammatory properties (Hashem *et al.*, 2023; Muthukrishnan *et al.*, 2022). The ability of endophytes to produce metabolites similar to that of its host is especially valuable as it may be an indication of either evolution or horizontal gene transfer. This is made easy because unlike most plant material it is easier to cultivate endophytes (Patil *et al.*, 2016). Through genome mining, a large number of gene clusters of endophytes are non-transcribed in lab, but special conditions can induce their transcription to produce Novel compounds (Tiwari and Bae, 2022; Zhang *et al.*, 2019; Wang and Mao, 2025).

1.5 Interaction of Endophyte and Plant

1.5.1 Symbiotic Interaction

The relationship of the endophytic fungi with the host can range from totally symbiotic to partly neutralistic. The fungus Improves specific properties to the plant: such as an increased rate of

growth, tolerance to stresses, diseases etc. In return fungi get protection and nutrient from the host (El-Metwally *et al.*, 2023; Mattoo and Nonzom, 2021; Latz *et al.*, 2018). Endophytes are known to enhance tolerance of the host to stresses such as drought, salinity, pathogens and heavy metal toxicities in both biochemical and physiological way (Zheng *et al.*, 2023; Hanaka *et al.*, 2021; Jia *et al.*, 2016).

1.5.2 Exchange of Metabolites

The interaction between two was favourable via this metabolite exchange mechanism. Metabolites produced by endophyte fungi would benefit the plants' defence mechanism, and chemical constituents within the plant exudate can induce gene expression of metabolic pathway in fungi (Gaikwad *et al.*, 2026; Zhang *et al.*, 2019; Wang and Mao 2025). This are two ways interaction that allow survival of the ecosystem under adverse condition (Islam *et al.*, 2026).

1.5.3 Ecological Importance

Endophytic fungi aid in promoting better plant health which in turn provides the resilience of the whole ecosystem, through effective nutrient recycling and environmental stress resistance (Ahmad *et al.*, 2026; Fite *et al.*, 2023). The role is often of increased importance in unfavourable nutritional or environmentally adverse locations (Du *et al.*, 2026; Sena *et al.*, 2024).

1.6 Rationale and Aim of Review

A major trend in natural product chemistry recently is the ever-increasing research interest on the natural products isolated from microbes and endophytes because of its potential in chemical diversity and medical properties (Atanasov *et al.*, 2021; Tiwari and Bae, 2022; Singh *et al.*, 2021). Advanced methods such as metabolomics, genome mining and AI are employed for the natural product discovery (Zhang *et al.*, 2019; Wang and Mao, 2025). There is increasing interest in the study of endophytic fungi from plants which thrive in biodiversity-rich areas and the diverse fungal metabolites from these areas (Gaikwad *et al.*, 2026; Mishra and Venkateswara Sarma, 2018). However, most of the endophytes are non-culturable. Moreover, the exact structures and mechanistic action of many compounds are unknown (Tiwari and Bae, 2022). Linking the genes that are present to their actual expression and product still remains a significant issue (Zhang *et al.*, 2019; Wang and Mao, 2025). The variation of metabolite expression in the lab environment poses significant reproducibility problems (Niepel *et al.*, 2019).

2. Endophytic Fungi Diversity and Source

The diversity in ecological niches occupied by endophytes indicates the extreme flexibility and ecological plasticity of endophytes (Zheng *et al.*, 2016; Ahmad *et al.*, 2026). The diversity of endophytes is dependent on host plant, environmental factors and habitats, and they were recognized as the most abundant microorganisms of plants (Zheng *et al.*, 2016; Selvakumar *et al.*, 2018).

2.1 Endophytes of Medicinal Plants

2.1.1 Medicinal Plants of Tropical Regions

As the tropical region possesses favourable climatic conditions such as high temperature, high humidity and abundant rainfall (El Mansy *et al.*, 2020; Bogas *et al.*, 2024), its medicinal plants

harbour one of the richest sources of endophytic fungi. High abundance of bioactive metabolites-producing fungi due to appropriate conditions in the tropics is also recorded (Bogas *et al.*, 2024). Novel alkaloid, terpenoids, polyketides and other compounds with significant activity against cancer and bacterial diseases, were derived from different structured tropical medicinal plants endophytes (Selvakumar *et al.*, 2018; Adeleke *et al.*, 2021). Host-endophyte co-evolution or gene transfer have been suggested due to structural comparison of fungal products to plant metabolites (Gaikwad *et al.*, 2026; Ramawat and Goyal, 2020).

2.1.2 Ethnomedicinal Plants

Endophytes inhabiting ethnomedicinal plants which are used in folk medicine are prime resources for isolation of biologically active compounds (Jia *et al.*, 2016; Adeleke and Babalola, 2021; Manganyi and Ateba, 2020). Hence, they serve as the hot spots of bioactive endophytes. Endophytic fungi isolated from ethnomedicinal plants exhibit strong antibacterial, antioxidant and cytotoxic activities. Drug discovery programmes of various potent compounds are obtained from these species (Selvakumar *et al.*, 2018). Therefore, research on bioprospecting of medicinally important plants in biologically diverse zones is actively pursued (Gaikwad *et al.*, 2026; Ibrahim *et al.*, 2021).

2.2 Endophytes of Crop Plants

2.2.1 Cereal

Varied communities of endophytic fungi were reported to occur in cereals such as rice, maize, wheat and barley. They support plants in better nutrient uptake, stress resistance and disease resistance (Zheng *et al.*, 2016; Malarvizhi *et al.*, 2023). Furthermore, certain cereal endophytes are known to synthesize active substances which possess anti-microbial and growth promoting properties, making them very important for sustainable agriculture (Bogas *et al.*, 2024; Masenya *et al.*, 2024).

2.2.2 Legumes

Endophytic microorganisms inhabiting legumes have attracted considerable interest due to their mutualism in nitrogen fixation, growth stimulation and increased plant defence against pathogen. The genus is famous due to production of medicinally potent active compounds (Jia *et al.*, 2016; Wijesekara and Xu, 2023). In legumes, endophytes have the ability to produce some secondary metabolites such as alkaloid and phenolic compounds which exhibit anti-oxidant and anti-microbial activities (Selvakumar *et al.*, 2018; Castronovo *et al.*, 2021).

2.2.3 Vegetables

Vegetables constitute an important reservoir for many different types of endophytic fungi; a large number of these are also consumed by humans. Endophytic fungi colonization in vegetable enhances its disease resistance and may improves post-harvest quality and shelf- life (Zheng *et al.*, 2016; Ling *et al.*, 2024). According to some recent reports, bioactive compounds of pharmaceutical interest are also synthesized by many vegetables, with potent anti-microbial and anti-inflammatory actions, thus giving it an edge for food processing and nutraceuticals (Selvakumar *et al.*, 2018; Gupta *et al.*, 2016).

Table 1: Endophytic Fungi, Host Plants, and Their Associated Bioactive Natural Products

Plant Host	Endophytic Fungus	Isolated Compound(s)	Bioactivity	References
<i>Camptotheca acuminata</i>	<i>Fusarium solani</i>	Camptothecin	Anticancer (Topoisomerase I inhibitor)	Banyal <i>et al.</i> , 2021; Varghese <i>et al.</i> , 2024
<i>Catharanthus roseus</i>	<i>Fusarium oxysporum</i>	Vinblastine & Vincristine	Anticancer (Microtubule destabilizers)	Swathi <i>et al.</i> , 2025; Varghese <i>et al.</i> , 2024
<i>Taxus spp.</i>	<i>Taxomyces andreanae</i> / <i>Alternaria sp.</i>	Paclitaxel (Taxol)	Anticancer (Microtubule stabilizer)	Tiwari & Bae, 2022; Banyal <i>et al.</i> , 2021
<i>Podophyllum spp.</i>	<i>Phialocephala fortinii</i>	Podophyllotoxin	Anticancer (Topoisomerase II inhibitor)	Banyal <i>et al.</i> , 2021; Varghese <i>et al.</i> , 2024
<i>Kandelia candel</i>	<i>Dothiorella sp.</i> ZJQQYZ-1	Phomosterol (7)	Anti-inflammatory (iNOS expression suppressor)	Li <i>et al.</i> , 2025
Cranberry (<i>Vaccinium macrocarpon</i>)	<i>Protoventuria sp.</i>	Naphthalenes polyketides	Antifungal	Rehman <i>et al.</i> , 2025
Mangrove plant	<i>Penicillium steckii</i> SCSIO 41025	Polyketide decalin derivatives	Antimicrobial	Chen <i>et al.</i> , 2021
<i>Ruprechtia salicifolia</i>	<i>Emericella nidulans</i> ATCC 38163	Emestrin	Anticancer	Prajapati <i>et al.</i> , 2025
<i>Ilex prenatal</i>	<i>Pestalotiopsis vismiae</i>	Pestalotic Acid	Anti-Inflammatory	Hwang <i>et al.</i> , 2025

2.3 Marine and Mangrove Endophytic Fungi

2.3.1 Marine Diversity

Marine ecosystems such as seaweeds, sea grasses and marine algae contain unique populations of endophytic fungi; a consequence of the high pressure and saline environment they inhabit (Zheng *et al.*, 2016; Behera and Das, 2023). The unique metabolic activities of the marine-derived endophytes can be attributed to its adaptation and hence leads to distinct secondary metabolites associated with this habitat only. In fact, marine endophytic fungi are being increasingly researched as novel sources of compounds that may have medicinal properties, as shown by its various anti-microbial, anti-cancer and anti-inflammatory applications (Bogas *et al.*, 2024; El-Bondkly *et al.*, 2021).

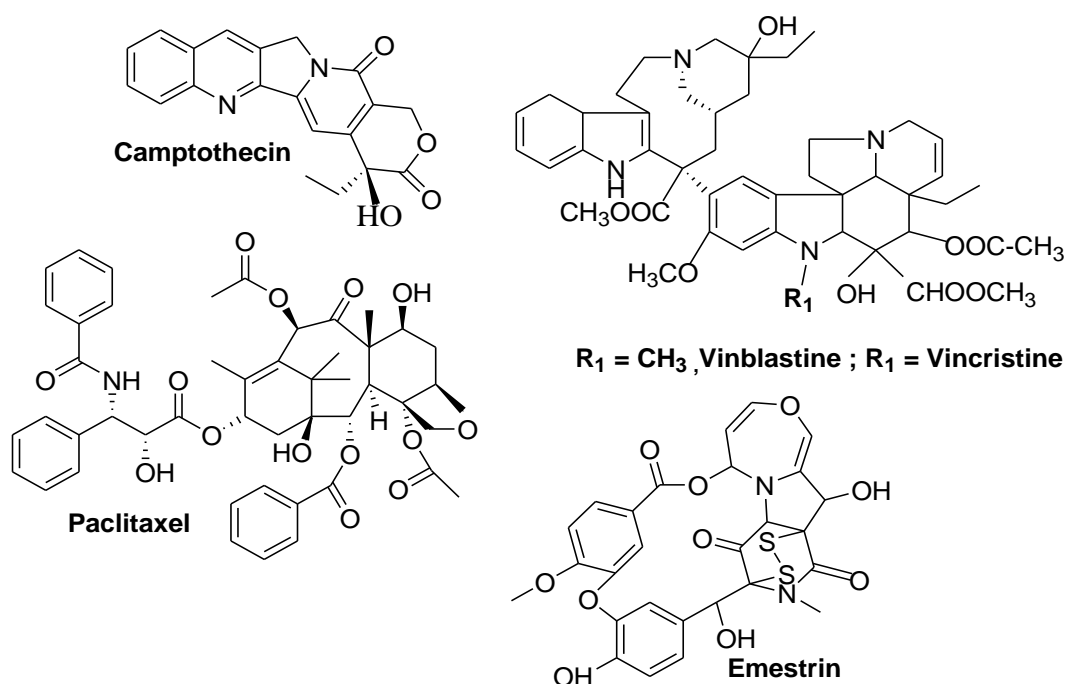


Figure 2: Some isolated compounds from endophytic fungi

2.3.2 Unique Metabolites

There is a wide range of bioactive secondary metabolites synthesized by marine and mangrove associated endophytic fungi, namely halogenated compounds, polyketides, and atypical alkaloids which often have profound pharmacological effects (Selvakumar *et al.*, 2018; Chen *et al.*, 2022). This higher bioactivity resulted from their ability to adapt severe condition with a high survival rate.

2.4 Environmental Effect on Diversity

2.4.1 Climate

Climate affect very significantly diversity of endophyte fungus, and, as expected for every organism that had its grow requirements depended by temperature and humidity, in tropic and subtropic region are isolated more diversity fungus (Bogas *et al.*, 2024). Seasonal variation of climate can have an influence on diversity and abundance and on metabolite generation (Zheng *et al.*, 2016; Wang *et al.*, 2024).

2.4.2 Host Specificity

Some endophytic fungi demonstrate a high level of specificity in the plants they inhabit, while others have the capacity to colonize a number of host species (Zheng *et al.*, 2016). The DNA of host plants and the constituent phytochemical profile of its tissue greatly influence the pattern and nature of colonization, and the subsequent chemical diversity within endophytic fungi.

2.4.3 Geographic Distribution

Location has a large effect on endophytic fungi diversity, the climate, type of soil and environment the plants are growing in all influence fungal variety (Zheng *et al.*, 2016; Selvakumar *et al.*, 2018). These are the areas in tropical rainforest and mangroves where highest diversity is formed but undiscovered areas like Africa and Asia represent a huge prospect of fungi as many unknown sites

from these regions harbour fungal species and possible medicine compounds (Gaikwad *et al.*, 2026; Wijerathna *et al.*, 2026).

3. Isolation and Culture of Endophytic Fungi

The process of isolation and culturing are essential procedures involved in all studies which aim to isolate natural products from endophytic fungi. The conventional procedure for this process usually relies on an aseptically prepared sample, whose surface has been sterilized. The samples should then be cultured on a growth medium which will be selected according to the species of fungi being studied (Selvakumar *et al.*, 2018; Du *et al.*, 2026).

3.1 Sample Collection and Handling

3.1.1 Plant Tissues

A number of different plant tissues can be sampled, including stems, roots, leaves, flowers and fruits. Generally, it is thought that healthy disease-free tissue is preferred for studies of endophytic fungi, but often a higher diversity can be gained by sampling different tissues on the same plant (Zheng *et al.*, 2016; Bogas *et al.*, 2024). Medicinal or ethnomedicinally important plants often have a higher percentage chance of yielding novel metabolites (Gaikwad *et al.*, 2026).

3.1.2 Sterile Transport

Once the samples have been collected, they should be placed inside a sterile plastic bag and transported to the lab. They should be kept chilled so the organism will not be damaged or its metabolic processes altered greatly. Ideally, these samples should be processed within 24 hours of being collected (Selvakumar *et al.*, 2018).

3.2 Surface Sterilization Techniques

Surface sterilization will eliminate the various epiphyte organisms found on the external tissues of the plants so that only the true endophytes will be isolated (Sahu *et al.*, 2022).

3.2.1 Ethanol Treatment

Treatment with 70-75% Ethanol will remove surface organisms and increase cell membrane permeability of the bacterial cell walls. Usually, 30 seconds to 2 minutes duration is utilized (Dos Reis *et al.*, 2022; Selvakumar *et al.*, 2018)

3.2.2 Sodium Hypochlorite Treatment

After treating with ethanol, the tissues are further washed with sterilization agents (5% Sodium hypochlorite) for 1-5 min in order to ensure the thorough surface sterilization of tissues (Zheng *et al.*, 2016).

3.2.3 Sterility Validation

To verify the success of surface sterilization, use the last wash water and place it on a growth medium. A lack of contamination shows success (Zheng *et al.*, 2016).

3.3 Isolation Techniques

3.3.1 Tissue Segment Plating

Take sterilized tissue segments and slice into smaller sections usually 1x1 cm and plate them on growth medium such as PDA and grown until the filaments appear on the media over a period of 3-14 days (Selvakumar *et al.*, 2018). The growing filaments will then be scraped off the plate and purified.

3.4 Culture Media and Growth Conditions

3.4.1 PDA (Potato Dextrose Agar)

Commonly used for the isolation of endophytic fungi and provides the most suitable combination of nutrients to allow growth (Selvakumar *et al.*, 2018).

3.4.2 MEA (Malt Extract Agar)

Commonly used and promotes sporulation which simplifies the recognition of the fungus (Senanayake *et al.*, 2020).

3.4.3 Czapek-Dox Agar

Often used for physiological studies as it is defined (Pandey *et al.*, 2024).

3.4.4 Growth Conditions

The most typical condition for endophytic fungi is 25-28 °C, kept in totally dark or low light, and generation time can range from 3-21 days, species dependent (Selvakumar *et al.*, 2018).

3.5 Storage of Isolates

3.5.1 Cryopreservation

Cryopreservation is arguably the most successful long-term method of storing an isolate, either in liquid nitrogen or at 8 °C. Cryoprotectants such as 10-20% Glycerol is added to the sample to inhibit the formation of ice crystals within the cells (Du *et al.*, 2026).

3.5.2 Slant Cultures

Alternatively, fungal cultures may be kept on agar slants stored at 4 °C. Although this method is economical, cultures need to be regularly sub-cultured to maintain their viability and a genetic drift may occur over time (Bogas *et al.*, 2024). Preservation is essential to ensure long term availability of fungal strains for metabolites production, bioactivity screening and molecular study (Selvakumar *et al.*, 2018).

4. Identification and Characterization of Endophytic Fungi

It is essential in natural product chemistry that the endophytic fungus is identified and characterized for taxonomic resolution, this allows a species to be linked with a metabolite that has been isolated from the fungus itself (Tiwari and Bae, 2022). In this context, utilize traditional morphological characterization along with modern omics approaches and molecular approaches are used to ensure correct identification and classification in a phylogenetic tree (Selvakumar *et al.*, 2018; Du *et al.*, 2026).

4.1 Morphological Characterization

Morphological identification is the most rudimentary, cost effective and preliminary approach when it comes to identifying endophytic fungi. Macroscopic and microscopic characteristics are examined visually which include colony colour, colony texture, colony growth rate, colony pigmentation, hyphae structure and spore morphology (Bogas *et al.*, 2024). Microscopic identification of fungal cultures would typically include a lactophenol cotton blue staining approach, in order to observe conidial structures, spores and hyphal characteristics (spore formation, spore morphology and septation). It is common practice not to consider morphological identification as a sufficient one on its own as the existence of cryptic species and the non-sporulating characteristic of fungal isolates is widespread (Zheng *et al.*, 2016).

4.2 Molecular Identification

At present, it is agreed that molecular methods are the most accurate and reliable for endophyte identification. Molecular identification relies on specific genetic markers which are employed in the identification of a specific endophytic fungus; of particular relevance is the use of internal transcribed spacer (ITS) regions of the ribosomal DNA. These sequences are favoured as there is a great deal of inter-specific variation between them, which aids species-level identification (Selvakumar *et al.*, 2018). The standard molecular methods for fungal identification involve the extraction of genomic DNA from pure cultures, then amplifying the ITS1-ITS4 regions of ribosomal DNA (rDNA) through polymerase chain reaction (PCR). After sequencing of the PCR product, the resulting sequence can then be compared with known sequences in well-established sequence databases (e.g., GenBank, UNITE) to determine species (Wagner *et al.*, 2018).

Molecular identifications methods have significantly refined our understanding of fungal taxonomy and dramatically expanded our knowledge on endophytic fungal diversity which had been severely underestimated in previous culture-dependent identification-based investigations (Du *et al.*, 2026).

4.3 Phylogenetic Analysis

The study of the phylogeny could provide information on evolutionary relationships between different classes of endophytic fungi. This involves alignment of DNA sequence data obtained (e.g. Alignment tools like Clustal W or MAFFT) followed by construction of a phylogenetic tree by neighbour-joining, maximum likelihood or Bayesian inference (Zheng *et al.*, 2016). Phylogenetic characterization of endophytic fungal communities revealed that members of the Ascomycota and Basidiomycota phyla dominate this niche. Furthermore, several unique phylogenetic clades can often be identified and appear to be specifically associated with host plant species or environmental condition (Harrison and Griffin, 2020). It is believed that host-plants might have co-evolutionary relationship with their endophytic species (Selvakumar *et al.*, 2018).

4.4 Advanced Omics Approaches

Omics sciences, which include genomics, transcriptomics and metabolomics are quickly becoming the tools that must be used in order to unravel the large potential of endophytic fungi (Wijayawardene *et al.*, 2022).

4.4.1 Genomics

The analysis of the full genome of an endophytic fungus will show the number of gene clusters that may code for the production of secondary metabolites; most endophytic fungi contain numerous gene clusters which may not be expressed until a particular stimulus has been applied (Zhang *et al.*, 2019; Wang and Mao, 2025).

4.4.2 Transcriptomics

Transcriptomic studies of gene expression profiles of endophytic fungi in response to particular stresses could not only inform genes encoding specific secondary metabolites but also the molecular basis for the organism's response to such environment (Du *et al.*, 2026).

4.4.3 Metabolomics

Metabolomic approaches including the analysis of secondary metabolites produced by endophytic fungi via GC-MS and LC-MS enabled the discovery and characterization of secondary metabolites. Accurate identification of the secondary metabolites with corresponding endophytic fungal species helps understand their biosynthetic pathways and the scope of secondary metabolites endophytic fungi can produce (Selvakumar *et al.*, 2018).

4.4.4 Multi-omics

The integration of the aforementioned three approaches with each other using Multi-omics is a powerful way of detecting novel compound that have been isolated from endophytic fungi (Zhang *et al.*, 2019; Wang and Mao, 2025).

4.5 DNA Barcoding and Next Generation Sequencing (NGS)

ITS regions coupled to the new generation of sequencing (NGS) method have revealed the composition of the communities of endophytes, without cultivation required. Metabarcoding has been used to prove the existence of fungi assumed to be non-culturable or rare, and the richness of endophytic community is more complex than expected (Du *et al.*, 2026)

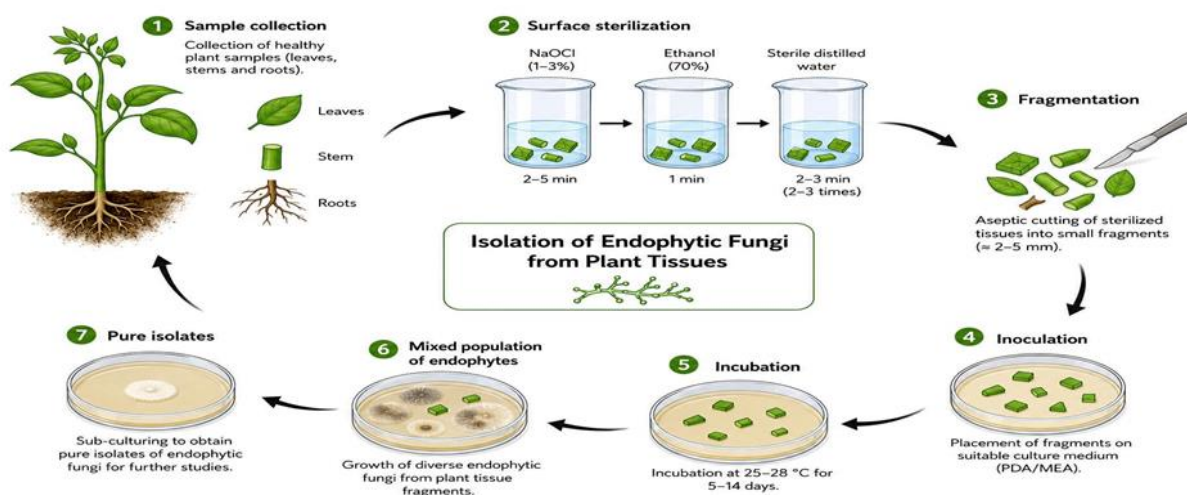


Figure 3: Isolation of endophytes

5. Extraction and Isolation of Secondary Metabolites

The producing abilities of the most abundant and chemically diverse of structurally related natural compounds from endophytic fungi is very high with extensive applications in medicinal fields,

recovery of these compounds requires well-optimized fermentation protocol and appropriate extraction and advanced chromatographic separation methods in natural product chemistry for correlation between fungal identity and bioactivity (Selvakumar *et al.*, 2018; Zhang *et al.*, 2019; Loukili *et al.*, 2022; Diass *et al.*, 2024; Wang and Mao, 2025).

5.1 Methods of Fermentation

5.1.1 Solid State Fermentation (SSF)

In this method, the endophytic fungi are cultivated on solid substrate with low water activity. The SSF is similar to in vivo conditions where fungi are resided inside the plant tissue and stimulate secondary metabolites production (Bogas *et al.*, 2024). SSF is well suited for testing varieties of endophytic fungi to search for potential bioactive compounds (Patil *et al.*, 2016)

5.1.2 Submerged Fermentation (SmF)

In SmF, fungi are grown in liquid culture medium, typically potato dextrose broth, malt extract broth, under controlled environment. Controlled environment, in SmF, leads to the possibility of large-scale metabolite production (industrial application) (Selvakumar *et al.*, 2018)

5.2 Extraction Methods

5.2.1 Solvent Extraction

The technique is the most widely applied methods to recover the fungal metabolites, where the appropriate solvents are employed according to their polarity. The commonly used solvents are Ethyl acetate, Methanol, Chloroform and Hexane (Meziane *et al.*, 2025; Gaikwad *et al.*, 2026). Ethyl acetate has been effective in extraction of most secondary metabolites from liquid fungal culture extract (Singh and Kumar, 2023).

5.2.2 Liquid-Liquid Partitioning

This is a separation technique for crude extracts in which compounds can be divided between two immiscible solvents (based on solvent polarity). Liquid-Liquid partitioning is often used as initial purification technique (Ingle *et al.* 2017; Wang and Mao, 2025).

5.2.3 Soxhlet and Maceration Techniques

Although Soxhlet or maceration methods are not widely utilized to extract compounds from the liquid cultures of fungi, these methods can efficiently be used for solid fungal biomass and dried culture materials so that complete extraction of secondary metabolites may occur (Bogas *et al.*, 2024).

5.3 Purification Techniques

5.3.1. Thin Layer Chromatography (TLC)

This is a basic and cheap technique to screen the crude extracts, gives an idea of the compounds present in the extract. Fractions can be selected for further purification steps conveniently (Kowalska and Sajewicz, 2022).

5.3.2 Column Chromatography

Column chromatography, the common technique is extensively used to separate the pure compounds from the crude extracts. The stationary phase is usually the silica gel. Elution takes

place with separation based on polarity of compound (Srivastava *et al.*, 2021; Selvakumar *et al.*, 2018). To obtain the separation gradient, elution is often utilized.

5.3.3 High Performance Liquid Chromatography (HPLC)

High resolution technique for the quantitative analysis of compounds used, among other things for the check of purity of obtained substances and for the identification standardization in natural product discovery (Zhang *et al.*, 2019; Wang and Mao, 2025).

5.3.4 Flash Chromatography and Preparative HPLC

They are efficient chromatography systems that permit quick separation and purification of bioactive molecules for more detailed analysis (Zhang *et al.*, 2019).

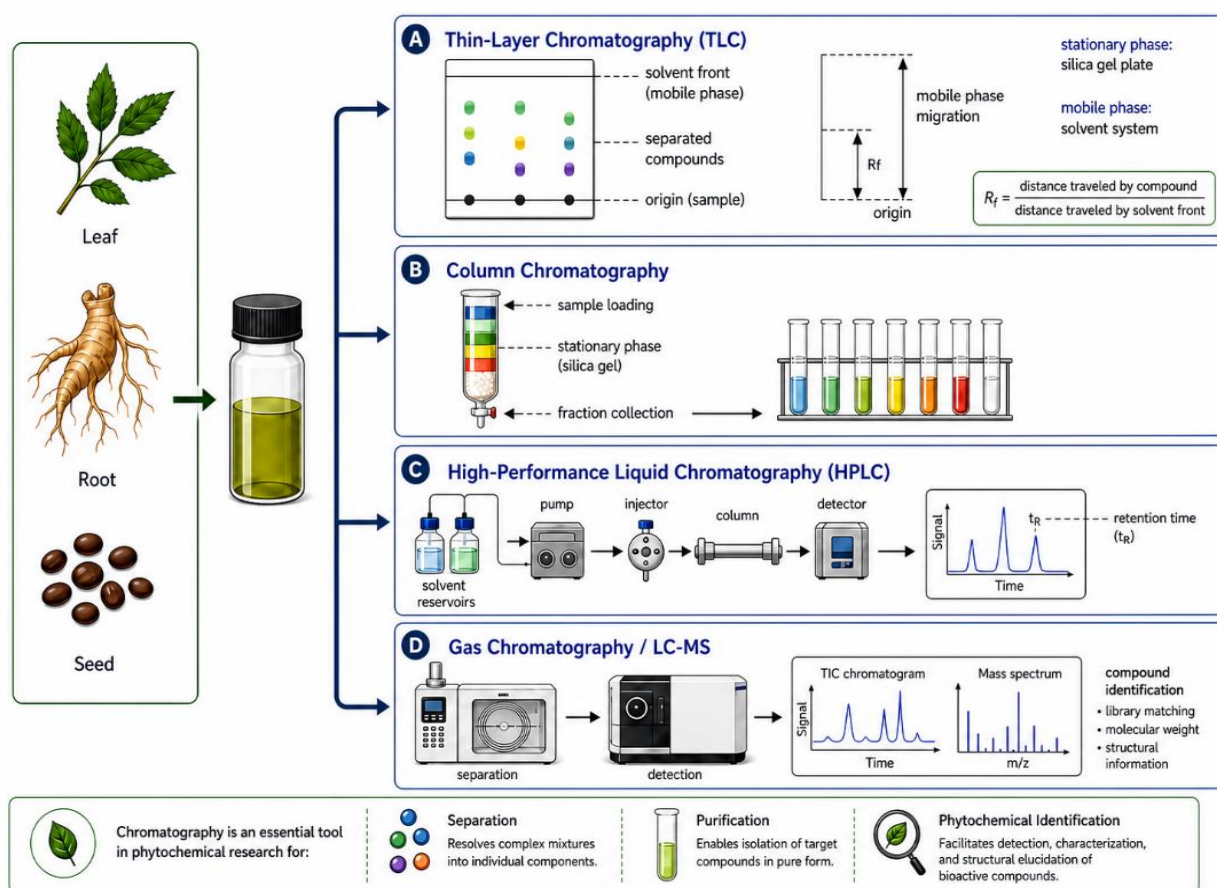


Figure 4: Chromatographic Techniques in Plant Extraction

5.4 Factors Influencing Metabolite Production

Secondary metabolites production is significantly affected by factors related to nutritional status and environmental condition (Yang *et al.*, 2018)

5.4.1 pH and Temperature

They depend upon the different species and their biological characteristics, typically within a pH of 5-7 and a temperature of 25-30°C. Alteration of these parameters from optimum value significantly changes the produced metabolites (Selvakumar *et al.*, 2018).

5.4.2 Nutrient Composition

The carbon and nitrogen sources used for metabolite production are the major nutrients. The yield of metabolites is often higher in complex media than in simple media. Synthesis of secondary metabolite is induced by limiting of nutrients (Bogas *et al.*, 2024).

5.4.3 Incubation Time

Maximum production of secondary metabolites occurs during stationary phase and extremely longer time of incubation may cause its decomposition or modification (Gaikwad *et al.*, 2026).

5.4.4 Co-culture and Stress Induction

New studies have shown that induction of abiotic stress, for example, salt, UV or certain chemical elicitors, or co-cultivation with other fungi leads to activation of dormant gene clusters responsible for the production of new metabolites (Zhang *et al.*, 2019; Wang and Mao, 2025).

5.5. Dereplication and Metabolite Profiling

The process of identifying known metabolites is referred to as dereplication. The available techniques are LC-MS/MS, molecular networking and database comparisons (e.g. GNPS) are useful to profile and determine known compounds quickly (Selvakumar *et al.*, 2018). These are essential in natural product discovery and to save the researchers from the re-isolation of known compounds with potential bioactivity.

6. Structural Characterization of Bioactive Metabolites

Structural Characterization involves identification, purity confirmation and stereo chemistry assessment of compounds derived from endophytic fungi. For structure elucidation, a combination of spectroscopic, chromatographic and crystallographic techniques is usually used (Selvakumar *et al.* 2018; Zhang *et al.*, 2019; Wang and Mao, 2025).

6.1 Spectroscopic Techniques

6.1.1 UV-Visible Spectroscopy (UV-Vis)

UV-Vis spectroscopy provides initial information on conjugated double bond systems and aromatic structure in a molecule. This technique is widely used to demonstrate the presence of chromophores group in the molecule (Lawan and Mustapha, 2026; Khan *et al.*, 2025).

6.1.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a powerful technique that provides information about functional groups present in the molecules of the fungal metabolite through the absorption pattern. Functional groups such as –OH, –C=O, –NH, aromatic ring etc., have characteristic absorptions and confirm the structure (Osunde *et al.*, 2024; Lawan and Mustapha, 2026).

6.2 MS Techniques

6.2.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS technique is extensively employed to characterize volatile and semi-volatile compounds, it provides the mass to charge ratio, fragment pattern and structure of compound (Li *et al.* 2023; Gaikwad *et al.*, 2026).

6.2.2 Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

LC-MS/MS technique is suitable for the detection and profiling of the non-volatile compounds; it aids in elucidating unknown compounds and to check for known compounds using techniques such as molecular networking and comparing the structure with databases (Huang *et al.*, 2023; Wang and Mao, 2025).

6.2.3 High Resolution Mass Spectrometry (HRMS)

HRMS produces highly precise masses enabling the unambiguous determination of molecular formulas of new compounds (Aalizadeh *et al.*, 2021).

6.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy has been found to be the most reliable technique in elucidation of structures of natural complex compounds (Lawan and Mustapha, 2026).

1D NMR (H and C NMR)

H NMR analysis elucidates the position of hydrogens in the molecule whereas C NMR technique gives detailed information about the carbon backbone of the molecule (Huang *et al.*, 2024; Selvakumar *et al.*, 2018).

2D NMR Techniques

The proton-proton couplings, correlation between protons and carbons and also proton and carbons connectivity in the molecules is determined using 2D techniques such as COSY, HSQC and HMBC (Bogas *et al.*, 2024).

NOESY/ROESY

They reveal information about the spatial arrangements and therefore determine the stereochemistry of the compound (Bogas *et al.*, 2024).

6.4 Chromatographic Methods to Ascertain Structure

The separation methods of Chromatography such as HPLC coupled with UV detection or a Diode Array Detector (DAD) were used to identify and authenticate compounds based on their characteristic spectral fingerprints (Zhang *et al.*, 2019; Wang and Mao, 2025).

6.5 X-ray Crystallography and Advanced Structural Techniques

X-ray crystallography has been accepted as the definitive tool for revealing precise 3D structure of molecules if a good crystalline structure can be obtained. This can lead to determination of the precise configuration and absolute configuration (Selvakumar *et al.*, 2018).

Other advanced techniques are:

- a) Circular Dichroism (CD) spectroscopy for analysis of chirality.
- b) Electronic Circular Dichroism (ECD) for determination of absolute configuration.
- c) Computational chemistry and docking to predict structure and evaluate biological activity.

6.6 An Integrated Approach to Structure Elucidation

Current structural analysis requires an integrated approach (using MS, NMR, IR, and UV) to ensure the structural identity (Zhang *et al.*, 2019; Wang and Mao, 2025).

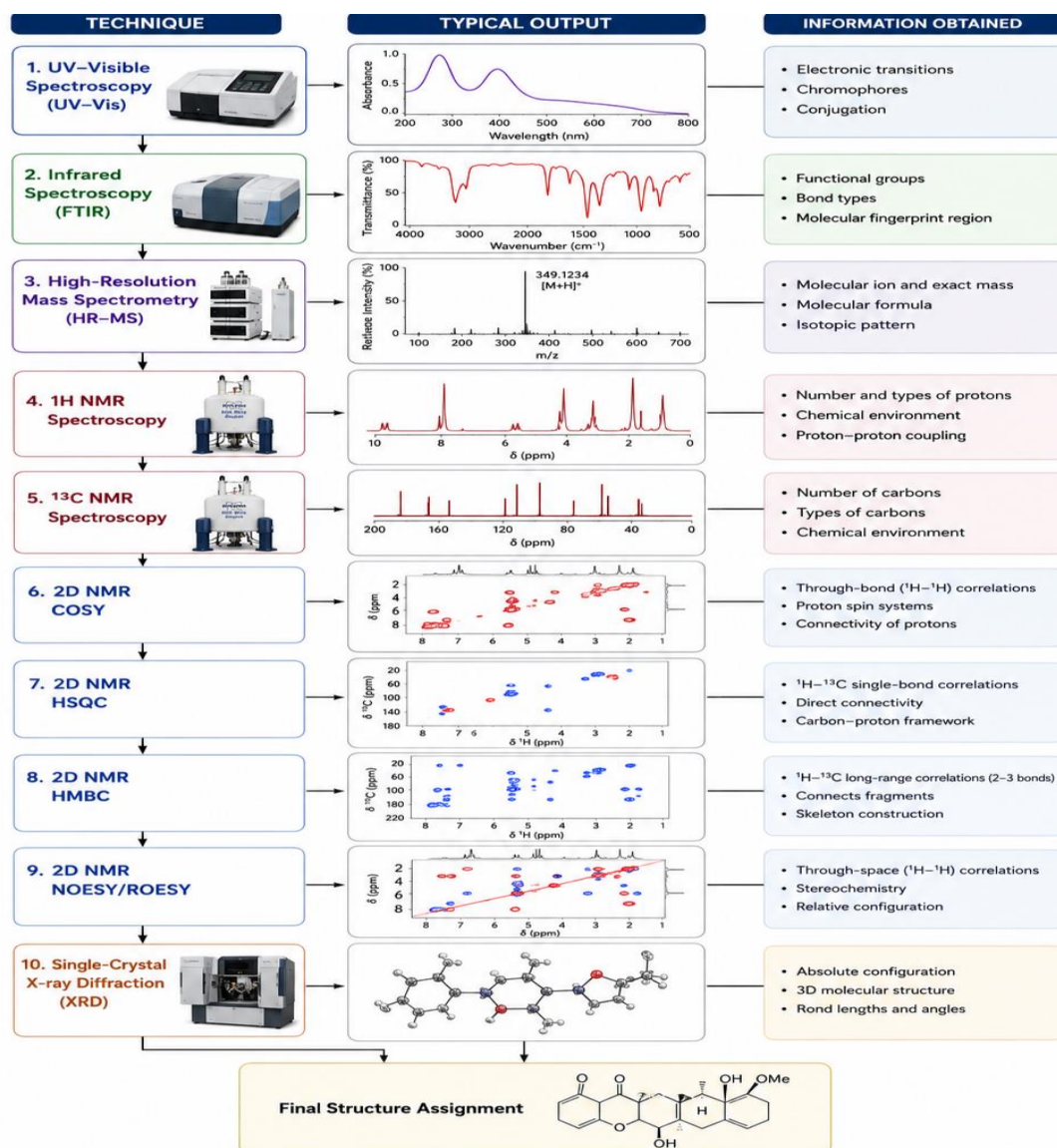


Figure 5: Spectroscopic Techniques for Structural Elucidation of Isolated Natural Products

7. Classes of Secondary Metabolites Produce by Endophytic Fungi

Endophytic fungi are a treasure trove of unique secondary metabolites that are very important in natural products chemistry and drug discovery. Most of the secondary Metabolites belong to the following classes and the class varies according to the metabolic pathways and the diverse bio-activities (Selvakumar *et al.*, 2018; Zhang *et al.*, 2019; Wang and Mao, 2025).

7.1 Alkaloids

This is a class of nitrogen-containing secondary metabolites commonly produced by endophytic fungi that have powerful pharmacological properties, including antitumor, antifungal and neurological effects. Usually, fungal alkaloids have very complex heterocyclic structure that make

them useful leads for medicinal chemistry applications (Gaikwad *et al.*, 2026). It has been known that the endophytic fungal alkaloids can interact with cellular receptors, Enzymes, DNA and therefore mediate their cytotoxic and antimicrobial activities. Structural diversity is common in fungal alkaloids since it arises from various metabolic pathways of fungal biosynthetic pathways (Selvakumar *et al.*, 2018).

7.2 Terpenoids

Terpenoids represent a huge class of fungal secondary metabolites, synthesized by isoprene units and widely dispersed among endophytic fungi, can vary between Mono-, Sesqui-, Di- and Triterpenoids, that possess varied bioactivities (Bogas *et al.*, 2024; Edder *et al.*, 2024). Strong anti-inflammatory, cytotoxic and antitumor activities are some of the bioactivities that can be obtained from terpenoids from endophytic fungi. In addition, terpenoids are also important signal molecules in host-fungi interaction which have been used extensively in eco systems (Zhang *et al.*, 2019; Wang and Mao, 2025).

7.3 Polyketides

Polyketides are a very diverse class of metabolites synthesized from polyketide synthase (PKS) metabolic pathways which are produced in abundance by endophytic fungi that exhibit antibiotic, cytotoxic and anti-inflammatory activity (Selvakumar *et al.*, 2018). Many compounds that possess important clinical activity such as cytotoxic and antibiotic agents have their structure derived from polyketides (Gaikwad *et al.*, 2026).

7.4 Phenolic Compounds

The phenolic compounds from endophytic fungi includes simple phenol structures to more complex polyphenols, some that exhibit bioactivities similar to flavonoids, are usually produced by endophytic fungi and they are best known for their antioxidant activity which is involved in free radical scavenging (Bogas *et al.*, 2024). The action of phenolics compounds is to counteract with destructive free radicals, which must be taken into careful consideration in treatment and prevention of chronic diseases such as cardiovascular disorders, cancer, and diabetes (Selvakumar *et al.*, 2018; Ouahbi *et al.*, 2024).

7.5 Peptides and Cyclic Compounds

Bioactive peptides, cyclic depsipeptides and non-ribosomal peptides can also be isolated from endophytic fungi and some of these compounds have been shown to exhibit antibiotic, antifungal and cytotoxic activity (Zhang *et al.*, 2019; Wang and Mao, 2025). Some non-ribosomal peptides are produced through Non-ribosomal Peptide Synthetase (NRPS) metabolic pathways and therefore have the characteristic features and potent bioactivity profiles expected from NRPS products (Gaikwad *et al.*, 2026).

7.6 Volatile Organic Compounds (VOCs)

Alcohols, ketones, esters and sulphur-containing compounds are examples of volatile organic compounds produced by endophytic fungi and these compounds are involved in interspecies interactions and communication (Bogas *et al.*, 2024). Antimicrobial and plant growth-promoting properties of fungal VOCs were reported which highlights potential application in agricultural and biotechnological settings (Selvakumar *et al.*, 2018).

7.7 Emerging and Unclassified Metabolites

A growing number of novel uncharacterized secondary metabolites are being identified from endophytic fungi by recent metabolomic and genome mining efforts. Many of these new metabolites are produced from cryptic biosynthetic gene clusters and can be activated upon induction by certain chemicals or environment factors (Zhang *et al.*, 2019; Wang and Mao, 2025). These new natural products are one frontier in natural products chemistry with potential for drug development and industrial use (Thomford *et al.*, 2018)

8. Pharmacological and Biological Activities of Endophytic Fungal Metabolites

Endophytic fungi, as producers of secondary metabolites, possess numerous pharmacological effects and show great promise for natural products chemistry and the development of pharmaceuticals. Such bioactivities may be a result of their diversity in structure, which enables interaction with more biological targets (Selvakumar *et al.*, 2018; Zhang *et al.*, 2019; Wang and Mao, 2025).

8.1 Antimicrobial Activity

Among many other bioactivities of secondary metabolites derived from endophytic fungi widely characterized is their antimicrobial properties towards a variety of bacterial and fungal groups and to some extent also towards viruses (Gaikwad *et al.*, 2026). In general, it has been reported that alkaloids, terpenoids, polyketides and peptides showed the antimicrobial activities through interfering with cell membranes, cell walls, or others metabolic ways to different extent that result in their antimicrobial actions. The recent development of multi-drug resistance bacteria has further motivated us to study the potent antibiotic activities of these microbial products (Selvakumar *et al.*, 2018).

8.2 Anticancer Activity

The secondary metabolites from endophytic fungi display distinct cytotoxic activity since these metabolites regulate pivotal cellular mechanisms including critical in controlling cell growth, regulating cell cycle progress, inducing apoptosis, targeting DNA and preventing angiogenesis (Zhang *et al.*, 2019; Wang and Mao, 2025). It has been reported that, the secondary metabolites of polyketides and alkaloids from the endophytic fungi display the strong activity in mediating the antitumor and anticancer features.

8.3 Antioxidant Activity

Phenolic compounds and other polyphenols isolated from several groups of endophytic fungi are reported to be potent antioxidants because of their capacity to scavenge free radicals. Since the compounds that can effectively scavenge free radicals play crucial roles in the prevention of numerous types of cellular damages (that are usually connected with chronic diseases such as cancer, diabetes, and cardiovascular problems), the phenolic compounds produced from fungi of various origins are of great importance (Bogas *et al.*, 2024; Selvakumar *et al.*, 2018). Antioxidant metabolites, which protect from oxidation damages that can be triggered by the development of chronic diseases like cancer, diabetes or cardiovascular diseases are significant factors (Muscolo *et al.*, 2024).

8.4 Anti-inflammatory Activity

The metabolic products from endophytic fungi possess significant anti-inflammatory activities by suppressing pro-inflammatory mediators like nitric oxide (NO), cytokines and cyclooxygenase (COX) enzymes (Selvakumar *et al.*, 2018). These activities indicate that these metabolites could have therapeutic applications in the treatment of inflammation like arthritis, asthma and neuroinflammatory disease.

8.5 Antidiabetic Activity

Some endophytic fungal metabolites possess antidiabetic potential by acting as inhibitors of carbohydrate hydrolysing enzymes like amylase-glucosidase (Gaikwad *et al.*, 2026), leading to decreased postprandial glucose levels and help in blood sugar control. Other metabolites enhance the insulin sensitivity and protect pancreatic-cells from oxidative damage (Zhang *et al.*, 2019; Wang and Mao, 2025).

8.6 Antiviral and Immunomodulatory Activities

Endophytic fungal metabolites have recently been reported for their antiviral activities against different viral strains. These could inhibit replication, entry into the host cell, protein synthesis of virus and many others (Selvakumar *et al.*, 2018). Also, these can play a part in immuno-modulatory activities by suppressing or activating the immune system as required for a treatment, thus it could be exploited for immunity-related diseases.

8.7 Other Biological Activities

Apart from the listed compounds, there are many other diverse bioactivities generated by endophytic fungi such as antiprotozoal activity, neuroprotection activity, hepatoprotection activity and cytoprotecting activity (Hashem *et al.*, 2023).

Antiprotozoal activity is defined as being against protozoa parasitic infection, neuroprotection as protection of the brain against neurodegenerative disorders. Hepatoprotection as against the damage on liver and cytoprotectant as defence of normal cells against toxic damages. The studies above prove the various kinds of bioactivities which compounds isolated from endophytic fungi possesses (Bogas *et al.*, 2024; Selvakumar *et al.*, 2018).

8.8 Mechanisms of Action

The biological activities of endophytic fungal metabolites have been achieved by diverse mechanisms such as:

- a) inhibition of enzyme activity
- b) binding and damage to DNA intercalation
- c) regulation of oxidative stress
- d) inducing apoptosis
- e) disturbance of microbial membranes
- f) regulating inflammatory signals pathway

Multi-target acting property will also help to increase their therapeutic effect and to avoid developing resistance (Zhang *et al.*, 2019; Wang and Mao, 2025).

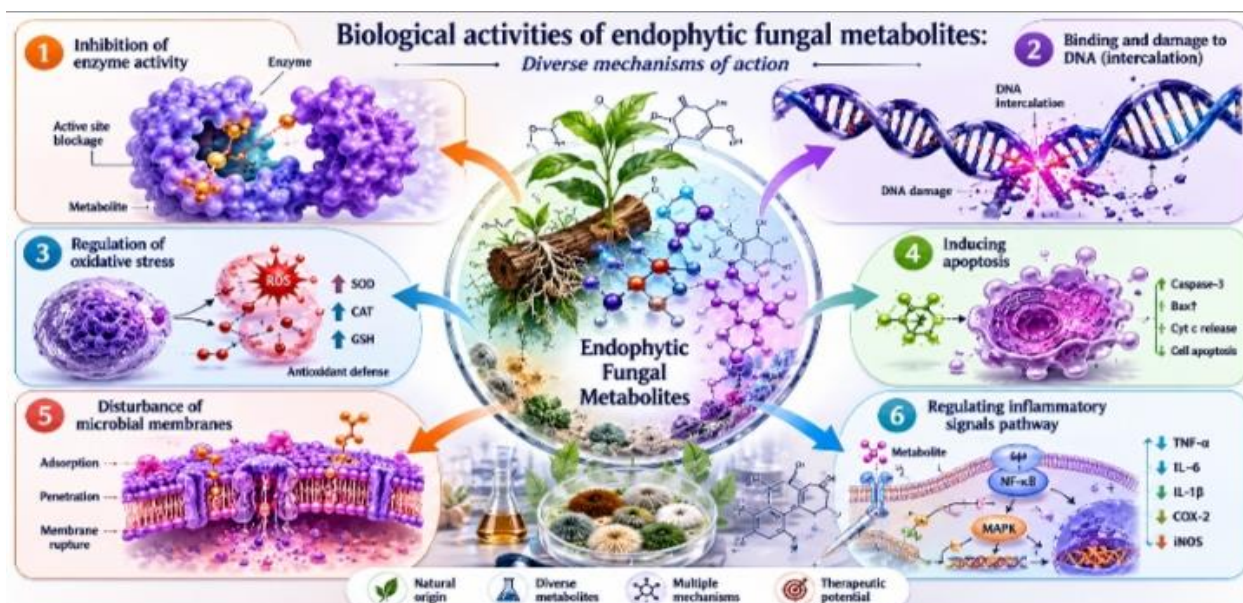


Figure 6: Mechanisms of action of endophytic fungal metabolites

8.9 Therapeutic Potential and Drug Development

Endophytic fungal metabolites offer potential resources for drug discovery and development. Due to the structural novelty and diversity of bioactivity, the endogenous fungal metabolites might be potential candidates to be used in lead optimization in pharmaceutical researches (Gaikwad *et al.*, 2026). Metabolomics, genome mining and synthetic biology accelerate the discovery and optimization of active compounds produced by endophytic fungi (Zhang *et al.*, 2019; Wang and Mao, 2025).

9. Industrial and Pharmaceutical Applications of Endophytic Fungi

Endophytic fungi are becoming a focus for valuable compounds and enzymes; they are known for a wide range of applications not only within the pharmaceutical industry but also in the area of agriculture, industry biotechnology and the environment (Selvakumar *et al.*, 2018; Du *et al.*, 2026). The wide range of structurally distinct secondary metabolites and enzymes produced by endophytic fungi make them attractive for sustainable bioprocessing and the drug-discovery sector (Selvakumar *et al.*, 2018; Du *et al.*, 2026).

9.1 Drug Discovery and Pharmaceutical Development

As a source of novel leads, endophytic fungi have gained increased attention for their potential to synthesize drugs with antimicrobial, anti-cancer, anti-inflammatory and antioxidant activities. Many of the molecules produced are structural precursors for drug discovery, and serve as an inspiration and template to be manipulated to achieve better pharmaceutical efficacy. The capacity of endophytic fungi to secrete molecules structurally similar to known pharmaceutical drugs, including alkaloids, terpenes and polyketides that originated from plants has been a breakthrough to minimize the reliance on either slow-growing or endangered medicinal plant resources (Gaikwad *et al.*, 2026). Modern drug discovery approaches like genome mining and metabolomics coupled with their implementation for increasing secondary metabolites production of endophytes has made them attractive target in this field (Selvakumar *et al.*, 2018).

9.2 Agricultural Applications

Sustainable agriculture can be greatly benefited from endophytic fungi as these microbes promote the growth of host plant and enhance their resistance to abiotic and biotic stresses through the provision of nutrients, synthesis of plant hormones and induction of defence mechanisms. In the arena of sustainable agriculture, they act as biocontrol agents for the pathogens of bacteria, fungi and nematodes and produce natural pesticides against plants diseases, thus reducing the reliance on chemical pesticides (Bogas *et al.*, 2024).

9.3 Food and Nutraceutical Applications

In food biotechnology the role of endophytes is to contribute valuable biomolecules such as natural antioxidants, preservative agents and flavour enhancing substances that increases the shelf life and the quality of food products (Selvakumar *et al.*, 2018). Similarly, health promoting dietary supplements like anticancer, antidiabetic and cardioprotective effects of some compounds isolated from endophytic fungi can have a profound influence on the nutraceutical sector (Zhang *et al.*, 2019; Wang and Mao, 2025).

9.4 Industrial Enzyme Production

Cellulases, amylases, proteases, lipases and xylanases are few examples of industrial enzymes that are produced by endophytic fungi. These enzymes are vital for the process industries namely food production, textiles, pulp, paper and fuel sector (Bogas *et al.*, 2024). This type of enzyme, synthesized by endosymbiotic fungi in ambient and moderate conditions, can therefore be considered superior to enzymes produced in other conditions, or even via chemical catalysis.

9.5 Environmental and Bioremediation Applications

Endophytic fungi have a role to play in the environmental science in degradation of pollutants and detoxification of polluted habitats. Heavy metals, hydrocarbons, and pesticides were biodegraded by some endophytic fungi (Du *et al.*, 2026). Some endophytic fungi also induce tolerance towards environmental stress factors and are capable of participating in the phytoremediation processes in disturbed ecosystems (Deng and Cao, 2017)

9.6 Nano Biotechnology Applications

The utilization of endophytic fungi for green synthesis of nano particles of silver, gold and zinc oxides is an emergent trend in nanobiotechnology that yielded nano-particles with antimicrobial, anti-cancer and catalytic activity (Selvakumar *et al.*, 2018). This biosynthesis technique is eco-friendly, cost effective and has great potential for scale-up.

9.7 Industrial Bioprocessing and Biotransformation

Endophytic fungi play a significant role in the biotransformation of inactive complex molecules into active/more bioavailable drugs which can be exploited for pharmaceutical applications and in the field of industrial bioprocessing (Zhang *et al.*, 2019; Wang and Mao, 2025). They are also useful in fermentation processes for the production of industrial organic acids, alcohol and bioactive intermediaries.

9.8 Synthetic Biology and Metabolic Engineering

Synthetic biology and metabolic engineering have contributed significantly to activate dormant biosynthetic gene cluster thereby producing desirable secondary metabolites from endophytic fungi (Zakariyah *et al.*, 2024). Synthetic biologists engineer biosynthetic pathways so that the biosynthesis of certain metabolites is amplified and this method is anticipated to lead an innovative approach for the application of endophytes in multiple industrial sectors in the next few years (Zhang *et al.*, 2019; Wang and Mao, 2025).

10. Challenges and Limitations of Endophytic Fungi Research

Despite the progress in the study of endophytic fungi and their secondary metabolites, a number of methodological and ecological problems as well as the scale-up difficulties have been identified which must be overcome if their complete application in natural products chemistry and biotechnology is to be realised (Selvakumar *et al.*, 2018; Du *et al.*, 2026).

10.1 Failure in Culturability and Difficulty in Cultivation

One major difficulty in the study of endophytes is the large percentage of endophytes which are not culturable in the standard culture media. The percentage is large enough that the total fungal diversity and metabolic capacity is substantially underestimated as the experimental condition cannot mimic those conditions within the plant (Zheng *et al.*, 2016). Even if the endophytic fungi are culturable, the production profile of metabolites will greatly differ from those produced within host plant (Selvakumar *et al.*, 2018).

10.2 Low Yield and Stability of Metabolites

The majority of endophytic fungi are very poor producers of their secondary metabolites and, thus very difficult to obtain in sufficient quantities for further analysis, for example, large-scale extraction. Besides this, the stability and rate of production of the metabolites produced are usually low and highly sensitive to minute changes in the pH, temperature, nutrient source and the other factors of cultivation (Bogas *et al.*, 2024).

10.3 Repeated Discovery of Known Compounds (Dereplication)

This is one of the persistent problems in natural products research. Research teams are discovering many known natural products repeatedly thereby leading to loss of time and effort, as well as increased costs of research programs (Zhang *et al.*, 2019; Wang and Mao, 2025). Tools like LC-MS/MS and molecular networking have become invaluable for the identification of already known metabolites thereby aiding dereplication, but this still remains a major issue while dealing with samples of endophytic fungi.

10.4 Complexity of Fungal–plant Interaction

It is well documented that the metabolic profile produced by endophytic fungi is highly influenced by the interaction inside the host plant, whereas the molecular mechanisms of this interaction are still largely unknown (Zheng *et al.*, 2016). It has been shown that *in vitro* cultures are unable to provide most plant derived signals and thereby cannot express some of their biosynthetic gene clusters (Polturak and Osbourn, 2021).

10.5 Complexity of Fungal Genome and Biosynthesis

Endophytic fungi possess large, complex genomes encoding many biosynthetic gene clusters that are silent. Many of these clusters are not active under typical laboratory conditions, thus many new compounds cannot be discovered (Zhang *et al.*, 2019; Wang and Mao, 2025). Although genome mining and epigenetic control can be used to induce clusters, inducing every possible cluster simultaneously remains an issue (Lee *et al.*, 2020).

10.6. Challenges of Industrial Production and Scale up

The challenge in transforming laboratory scale fungal metabolites production into industrial scale application has been faced by scientists for a long time now, where production of such compounds in large scale is a very difficult task due to low yield, chances of contamination, production variability (Selvakumar *et al.*, 2018). Optimization of fermentation conditions for the scaled-up production is also labour and time consuming and often species-specific.

10.7 Standardisation and Reproducibility Issues

The variation observed during research of endophytic fungi can also be attributed to the lack of standardization in protocols for isolation of endophytic fungi, culture methods and extraction techniques (Bogas *et al.*, 2024). Differences in methodology between different research groups do not allow for comparative results between laboratory works thereby causing ambiguity in findings.

10.8 Regulatory and IP Issues

There are number of regulatory issues and questions concerning the commercial development of endophyte produced compounds. Patentability of compounds derived from natural sources has to be dealt with, as well as the question of ownership of resources extracted from plants used in traditional medicine (Du *et al.*, 2026).

11. Future Perspectives

Although the investigation of endophytic fungi has made a great step forward in the past decade, their application of natural products chemistry, biotechnology and medicine have not fully explored. It is envisioned that research will involve integration of high-tech omics technologies, synthetic biology and computational methods in the future to unlock the latent metabolic potential of these microbes (Selvakumar *et al.*, 2018; Du *et al.*, 2026).

11.1 Genome Mining and Silent Gene Cluster Activation

The most promising future direction includes the mining of genomes to look for biosynthetic gene clusters (BGCs) involved in the synthesis of secondary metabolites. Many of the endophytes have silent or cryptic BGCs that are not expressed under conventional laboratory culture conditions (Zhang *et al.*, 2019; Wang and Mao, 2025). A variety of strategies like epigenetic modification, co-culture, chemical elicitation are being developed to activate silent gene clusters to access a whole new range of novel compounds with unusual scaffolds (Gaikwad *et al.*, 2026).

11.2 Integration of Multi-Omics Approaches

Integration of genomics, transcriptomics, proteomics and metabolomics is believed to revolutionize the way endophytes are studied. These multi-omics approaches facilitate an integrated analysis of

gene-metabolite relationship and biosynthetic pathways (Zhang *et al.*, 2019; Wang and Mao, 2025). Application of systems biology approach will greatly enhance the chances of identification of novel bioactive compounds. It would improve the prediction of metabolic capabilities of fungal endophytes.

11.3 Applications of Artificial Intelligence and Machine Learning

AI and ML are powerful and essential techniques for rapid natural product discovery and can be applied to the following areas:

- a) Prediction of bioactivity of compounds
- b) Prediction of BGCs related to metabolite biosynthesis
- c) Analysis of extensive metabolomic datasets to rank the fungal strains to be screened for bioactivity

Combined usage of AI with genomic data sets and metabolomic data can lessen the redundancy in natural products discovery (Selvakumar *et al.*, 2018).

11.4 Synthetic Biology and Metabolic Engineering

The most potential field in the redesigning and optimization of metabolic pathways of endosymbiotic fungi is Synthetic Biology. Genetic engineering has enhanced production yield and also activated silent pathways. Heterologous expression of BGCs can also be achieved which is beneficial for industrial applications and improved commercial value (Du *et al.*, 2026).

11.5 Sustainable Bioprospecting and Biodiversity Conservation

Sustainable bioprospecting techniques must be implemented, especially from regions like tropics and mangroves which are rich in biodiversity. Preservation of host plants and fungal habitat should also be an important goal to maintain fungal diversity and to prevent loss of useful fungal resources (Zheng *et al.*, 2016). Moreover, appropriate framework for bioprospecting and mechanism for benefit sharing needs to be formulated.

11.6 Clinical Translation and Drug Development

In vitro evaluation of many metabolites from endophytic fungi have shown promising activities; but only few have reached clinical trials. Validation of these studies through Preclinical validation, toxicological studies, pharmacokinetic and pharmacodynamic analysis of drug compounds, drug formulation and delivery systems, is critical to overcome the long and arduous path from laboratory to clinic (Selvakumar *et al.*, 2018).

11.7 Nanotechnology and Advanced Delivery Systems

Nanotechnology is expected to offer a boost in enhanced bioavailability and therapeutic efficacy of fungal metabolites. Nanocarriers can improve stability, solubility and targeted delivery of these drugs (Zhang *et al.*, 2019; Wang and Mao, 2025).

12. Conclusion

Endophytic fungi are valuable and unexplored resources of novel bioactive secondary metabolites with significant potential as potential drug candidates, in agriculture and biotechnology industries. Diversity of these fungi has increased and silent BGCs that can produce useful natural products are now becoming readily available due to advancements in molecular techniques and omics

technologies. There are several other biological activities for fungal endophytes' metabolites which include antibacterial, anticancer, anti-oxidant, anti-inflammatory, anti-diabetic which are useful as leads for drugs. Apart from pharma, these fungi can also find use in sustainable agriculture, bioremediation and production of industrial enzymes. Nevertheless, difficulty in culturing, unstable yields of compounds and dereplication issues are hindrances to full utilization of endophytic fungi resources, but integration of synthetic biology, metabolic engineering, AI and high throughput screening technologies along with sustainable bioprospecting techniques can overcome these limitations to achieve more from endophytic fungi.

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