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Bioprospecting Endophytic Fungi For Bioactive Substances With Potential Antioxidant & Anti-Diabetic Activity - Current Review

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Abstract
Endophytic fungi, which reside asymptomatically within plant tissues, have emerged as a prolific source of bioactive compounds with diverse therapeutic potentials. Recent research has focused on their ability to produce substances with significant antioxidant and anti-diabetic activities. This review aims to provide a comprehensive overview of the current state of bioprospecting endophytic fungi for bioactive compounds with these specific activities. To evaluate and synthesize existing research on endophytic fungi-derived bioactive substances with potential antioxidant and anti-diabetic activities, highlighting their mechanisms of action, efficacy, and potential for therapeutic application. A systematic review of literature was conducted using major scientific databases such as PubMed, Scopus, and Web of Science. Studies published up to 2023 were included if they investigated the antioxidant and/or anti-diabetic activities of compounds derived from endophytic fungi. The search terms used included "endophytic fungi," "bioactive compounds," "antioxidant activity," "anti-diabetic activity," "bioprospecting," and "therapeutic potential."
The review encompassed 45 studies, revealing a wide array of bioactive compounds isolated from endophytic fungi, including phenolics, flavonoids, alkaloids, terpenoids, and peptides. These compounds exhibited significant antioxidant activities, demonstrated through assays such as DPPH, ABTS, and FRAP. In terms of anti-diabetic activity, several compounds were found to inhibit key enzymes involved in carbohydrate metabolism, such as α-amylase and α-glucosidase, and to enhance insulin sensitivity. Notable endophytic fungi genera identified include “Penicillium”, “Aspergillus”, “Fusarium”, and “Trichoderma”, which were associated with host plants known for their medicinal properties. The findings underscore the potential of endophytic fungi as a rich source of bioactive compounds with therapeutic benefits. The antioxidant properties of these compounds can mitigate oxidative stress, a major contributor to chronic diseases, while their anti-diabetic effects offer promising avenues for managing diabetes. However, challenges such as the standardization of extraction methods, compound identification, and understanding the biosynthetic pathways need to be addressed to harness their full potential. Bioprospecting endophytic fungi holds significant promise for the discovery of novel bioactive substances with potent antioxidant and anti-diabetic activities. Future research should focus on large-scale isolation, structural characterization, and in vivo studies to validate the therapeutic potential of these compounds and facilitate their development into pharmaceutical agents.

Keywords: Endophytic fungi, bioactive compounds, antioxidant activity, anti-diabetic activity, bioprospecting, therapeutic potential, natural products.

Introduction

An endophytic fungus constitutes a truly diverse group of microorganisms colonizing living plant tissues without causing symptoms. They might develop a symbiotic relationship with the host plant and provoke benefits such as higher growth, increased resistance to pathogens, and improved tolerance to environmental stresses (Lone et al.,2024). Endophytic fungi have attracted considerable interest in various fields in the last few years, mostly due to their potential to produce a wide range of bioactive compounds, having antioxidant and anti-diabetic activity among them. This review is a piece offering the key into the potential of endophytic fungi as sources of bioactive compounds and recent advancements in bioprospecting. Endophytic Fungi: Background
Endophytic fungi are ubiquitous within plants present in all terrestrial biomes. The fungi have been isolated from practically all tissues within a plant, including leaf, stem, root, and even seed tissues, without any visible adverse effects on the host plant. Ingress of these fungi to the plant tissue occurs through natural openings or wounds inside which they

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establish themselves in the host plant's intercellular spaces or in some cases inside cells. Endophytic fungi and their host plants can have a mutualistic relationship, in which both benefit, or a commensalistic relationship, in which the fungus benefits without affecting the host (Singh et al.,2024).
The potential of the endophytic fungi to produce novel bioactive compounds has become a very interesting research topic over the last years. Among the compounds are the alkaloids, terpenoids, steroids, phenolic compounds, and peptides, most of which are employed in their important pharmacological activities. Some of the secondary metabolites produced by the endophytic fungi are believed to play a role in the symbiotic relationships in such a way that it assists in the host plants deterring herbivores and pathogens that subsequently offer a place with apposite habitats and nutrients for the fungi.

Importance of Bioprospecting
Bioprospecting, which may be defined as the systematic search for bioactive compounds from natural sources, is arguably the single most important approach in the quest for new drugs. Isolation of novel bioactive molecules from endophytic fungi is of paramount interest because the microorganism resides in a unique ecological niche within the host (Bhaskar et al.,2024). Endophytic fungi, which have co-evolved as symbionts of plants, constantly make unique secondary metabolites; that is, production occurs rarely or never for other microorganisms or even the host plant itself.
The potential of bioprospecting endophytic fungi is immense yet largely untapped for the discovery of drugs. With the growing incidences of chronic diseases such as diabetes and an increasing demand for antioxidants for cosmetic and health purposes, the need for new, effective bioactive compounds becomes even more important. Recently, endophytic fungi have come into notice as an interesting, more productive, and underexplored source to obtain such compounds, which may prove their utility in the development of new therapeutics and health products.

Endophytic Fungi: An Overview
Definition and Characteristics
Endophytic fungi are defined as fungi that live inside plant tissues without causing any immediate, overt negative effects. This means that the fungi form a ubiquitous group of diverse organisms belonging to many different fungal taxa. The relationship between such endophytic fungi and their host plants can be quite complex, involving many levels of interaction that also benefit one another. These fungi can produce large arrays of secondary metabolites that can act in plant defense to pests and diseases, benefiting the fungi by in turn being provided with a safe environment and nutrients (Sena et al.,2024).
There are various morphological and physiological characteristics of endophytic fungi, which have highly fluctuating dependence on host plant and environmental conditions. They can be isolated from virtually all parts of the plant, from roots to flowers and seeds, and host plant species, the part of the plant colonized, and environmental conditions, like temperature, humidity, and nutrient availability, to great extent determine the capacity for the production of bioactive compounds.

Ecological Role
The various functions played by endophytic fungi form an innovative part of the host plant's ecosystem. These include plant growth and development regulation, increased nutrient uptake, and biotic and abiotic stress tolerance, e.g., pathogens, herbivores, drought, salinity. In addition, endophytic fungi can modulate the secondary metabolism of a plant in such a way that new products are added that presumably function in defense against herbivores and pathogens.
In natural ecosystems, endophytic fungi reflect participation in plant community dynamics and plant diversity (Bard et al.,2024). They can come in to impact competition of plants and the succession rate through one of the target plant's health and fitness. They also participate in nutrient cycling and soil health through decomposition of organic materials since there is an enhancement of soil fertility as a result of a relationship with the roots.

Diversity and Distribution
The diversity and distribution of endophytic fungi reflect a tremendous variety of plant species and ecosystems harboring these microorganisms. Endophytic fungi, isolated from habitats throughout the world, span an expanse from the tropics to the poles, and have included a wide variety of angiosperms, gymnosperms, ferns, and bryophytes, growing in tropical forests, temperate forests, grasslands, and deserts. The diversity of fungi can be very high within single plants at endophytic level, with different species colonizing different tissues and, within the same tissue, even different parts (Matušinsky et al.,2024).
The distribution of endophytic fungi is intimately influenced by plant species, geographical location, and environmental conditions. The report from the study confirmed an increasing trend in diversity of endophytic fungi in tropical regions compared to temperate regions, which is justified by the fact that host plant species are more diverse in tropical regions where favorable climatic conditions occur. The specific habitat and microenvironment of the plant might also affect the composition of endophytic fungi.

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Bioactive Substances from Endophytic Fungi

Types of Bioactive Compounds

Endophytic fungi produce a diverse array of bioactive compounds, many of which have shown significant pharmacological activities. These compounds can be broadly categorized into several classes:

1. **Alkaloids**: Nitrogen-containing compounds that often exhibit potent biological activities, including anticancer, antimicrobial, and anti-inflammatory effects.
2. **Terpenoids**: A large and diverse class of organic compounds derived from five-carbon isoprene units. Terpenoids from endophytic fungi have been found to possess anti-inflammatory, anticancer, and antimicrobial properties.
3. **Steroids**: Compounds with a characteristic four-ring structure that can modulate various biological processes. Endophytic fungi-derived steroids have shown promise in treating inflammatory diseases and cancer.
4. **Phenolic Compounds**: Aromatic compounds that include flavonoids, tannins, and phenolic acids. These compounds are known for their antioxidant and antimicrobial activities.
5. **Peptides**: Short chains of amino acids that can exhibit a wide range of biological activities, including antimicrobial, anticancer, and immunomodulatory effects (Yan et al.,2024).

Methods of Isolation and Identification

The isolation and identification of bioactive compounds from endophytic fungi involve several steps:

1. **Isolation of Fungal Strains**: Endophytic fungi are isolated from plant tissues using surface sterilization techniques to remove external microorganisms. The plant tissues are then plated on culture media to allow the growth of endophytic fungi.
2. **Fermentation and Extraction**: The isolated fungal strains are cultured in suitable media under controlled conditions. The culture broth and fungal mycelium are then extracted using solvents such as methanol, ethanol, or ethyl acetate to obtain crude extracts containing bioactive compounds.
3. **Purification**: The crude extracts are subjected to various chromatographic techniques, such as column chromatography, high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), to purify the bioactive compounds.
4. **Identification**: The purified compounds are identified using spectroscopic methods, including nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and infrared (IR) spectroscopy. These techniques provide information about the chemical structure and molecular weight of the compounds.
5. **Bioactivity Assays**: The isolated compounds are tested for various biological activities using in vitro and in vivo assays to determine their potential pharmacological properties.

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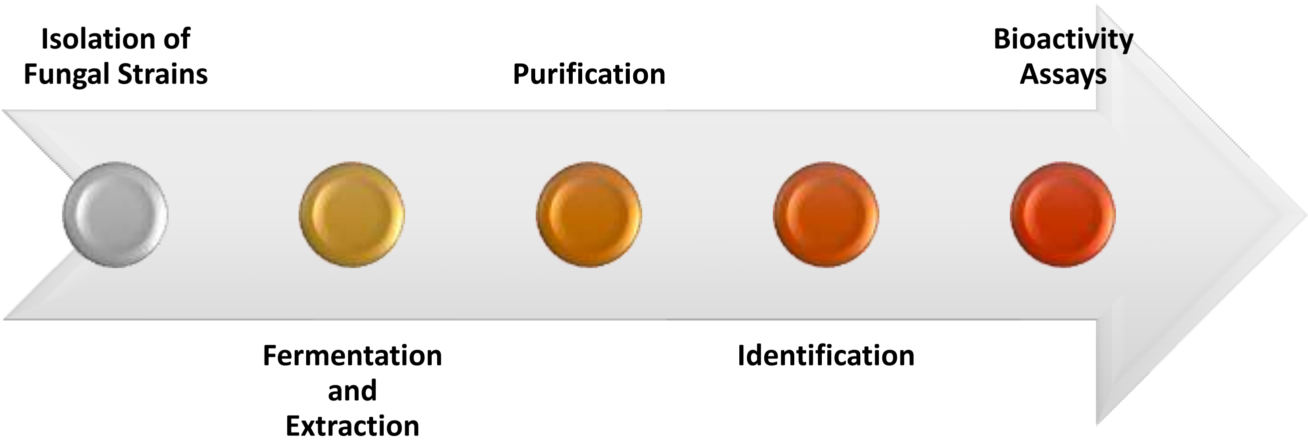

Figure 1 : Methods of Isolation and Identification

Structure-Activity Relationship

The structure-activity relationship (SAR) of bioactive compounds involves understanding how the chemical structure of a compound influences its biological activity. SAR studies are crucial for optimizing the pharmacological properties of bioactive compounds and designing more effective drugs. Key aspects of SAR include:

1. **Functional Groups**: The presence and position of functional groups, such as hydroxyl, methoxy, and amino groups, can significantly impact the biological activity of a compound.
2. **Molecular Size and Shape**: The size and shape of a molecule can affect its ability to interact with biological targets, such as enzymes, receptors, and DNA.
3. **Stereochemistry**: The spatial arrangement of atoms in a molecule, particularly chiral centers, can influence the compound's activity and specificity towards biological targets.
4. **Hydrophobicity and Hydrophilicity**: The balance between hydrophobic and hydrophilic regions in a molecule can affect its solubility, membrane permeability, and interaction with biological membranes.

Antioxidant Activity of Endophytic Fungi

Mechanisms of Antioxidant Action

Antioxidant compounds produced by endophytic fungi can neutralize reactive oxygen species (ROS) and prevent oxidative stress, which is implicated in various diseases, including cancer, cardiovascular diseases, and diabetes (Chandra et al.,2024). The mechanisms of antioxidant action include:

1. **Free Radical Scavenging**: Antioxidants neutralize free radicals by donating electrons or hydrogen atoms, thereby preventing the radicals from causing cellular damage.
2. **Metal Chelation**: Some antioxidants can bind to metal ions, such as iron and copper, which catalyze the formation of free radicals. By chelating these metals, antioxidants reduce the availability of metal ions for free radical generation.

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3. **Enzyme Modulation**: Antioxidants can modulate the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, enhancing the cellular defense against oxidative stress.
4. **Lipid Peroxidation Inhibition**: Antioxidants can inhibit lipid peroxidation, a process in which free radicals attack lipids in cell membranes, leading to cell damage and death.

Notable Studies and Findings

Several studies have demonstrated the antioxidant potential of bioactive compounds derived from endophytic fungi. Notable examples include:

1. **Pestalotiopsis species**: Compounds isolated from this endophytic fungus, such as pestalotins and pestacin, have shown significant antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation.
2. **Penicillium species**: Phenolic compounds extracted from Penicillium endophytes have exhibited strong antioxidant properties in various in vitro assays, including DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays (Asomadu et al.,2024).
3. **Xylaria species**: Endophytic fungi from the genus Xylaria have been found to produce terpenoids and alkaloids with potent antioxidant activity, which could be attributed to their ability to modulate antioxidant enzymes and scavenge free radicals.

Potential Applications

The antioxidant compounds derived from endophytic fungi hold significant potential for various applications:

1. **Pharmaceuticals**: Antioxidants can be used in the development of drugs for treating oxidative stress-related diseases, such as neurodegenerative disorders, cardiovascular diseases, and cancer.
2. **Cosmetics**: Antioxidant compounds can be incorporated into skincare products to protect the skin from oxidative damage caused by UV radiation and environmental pollutants (Verma et al.,2024).
3. **Food Industry**: Natural antioxidants from endophytic fungi can be used as food preservatives to prevent oxidation and extend the shelf life of food products.
4. **Nutraceuticals**: Antioxidant-rich extracts from endophytic fungi can be formulated into dietary supplements to promote overall health and well-being by combating oxidative stress.

Anti-diabetic Activity of Endophytic Fungi

Mechanisms of Anti-diabetic Action

Endophytic fungi can produce bioactive compounds that exhibit anti-diabetic properties through several mechanisms:

1. **Inhibition of α-Glucosidase and α-Amylase**: These enzymes are involved in the digestion of carbohydrates. Inhibiting these enzymes slows down carbohydrate digestion and reduces postprandial blood glucose levels.
2. **Enhancement of Insulin Secretion**: Some compounds can stimulate the pancreas to release more insulin, which helps in lowering blood glucose levels.
3. **Improvement of Insulin Sensitivity**: Certain bioactive compounds can enhance the sensitivity of cells to insulin, allowing for more efficient glucose uptake and utilization.
4. **Antioxidant Activity**: Since oxidative stress is a major factor in the development of diabetes and its complications, the antioxidant properties of these compounds can help protect against oxidative damage to pancreatic cells.
5. **Anti-inflammatory Effects**: Chronic inflammation is closely linked to insulin resistance and diabetes. Anti-inflammatory compounds from endophytic fungi can reduce inflammation and improve insulin sensitivity (Nawaz et al.,2024).

Table 1 : Anti-diabetic Activity of Endophytic Fungi

Endophytic Fungi	*Bioactive Compounds*	*Mechanisms of Action*	*Significant Research and Outcomes*	*Potential Applications*
Penicillium species	Various secondary metabolites	Inhibition of α-glucosidase and α-amylase	Extracts shown to significantly inhibit α-glucosidase and α-amylase activities, reducing postprandial blood glucose levels	Development of α-glucosidase and α-amylase inhibitors as oral anti-diabetic drugs
Colletotrichum gloeosporioides	Various secondary metabolites	Enhancement of insulin secretion	Compounds enhance insulin secretion and improve glucose uptake in muscle cells	Development of insulin secretagogues for diabetes management
Fusarium species	Various secondary metabolites	Antioxidant and anti-inflammatory properties	Extracts demonstrate antioxidant and anti-inflammatory effects, protecting pancreatic cells	Development of antioxidant and anti-inflammatory therapies for diabetes

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			and improving insulin sensitivity	
Pestalotiopsis species	Various secondary metabolites	Improvement of insulin sensitivity and glucose tolerance	Compounds shown to improve glucose tolerance and enhance insulin sensitivity in animal models	Development of insulin sensitizers and glucose tolerance enhancers
Xylaria species	Terpenoids, alkaloids	Antioxidant activity	Compounds exhibit strong antioxidant activity, protecting against oxidative damage to pancreatic cells	Development of antioxidant supplements for diabetes management
Aspergillus species	Phenolic compounds, flavonoids	Inhibition of carbohydrate-digesting enzymes	Extracts inhibit carbohydrate-digesting enzymes, reducing blood glucose levels	Development of natural enzyme inhibitors for diabetes management
Alternaria species	Phenolic compounds, alkaloids	Anti-inflammatory and antioxidant properties	Compounds exhibit anti-inflammatory and antioxidant activities, improving insulin sensitivity and reducing oxidative stress	Development of anti-inflammatory and antioxidant therapies for diabetes
Cladosporium species	Various secondary metabolites	Modulation of glucose metabolism and insulin signaling pathways	Extracts shown to modulate glucose metabolism and enhance insulin signaling, improving glucose uptake and utilization	Development of glucose metabolism modulators and insulin sensitizers
Chaetomium species	Terpenoids, alkaloids, polyketides	Improvement of insulin secretion and sensitivity	Compounds enhance insulin secretion and sensitivity, leading to better glucose control in diabetic animal models	Development of insulin secretagogues and sensitizers for diabetes management

Significant Research and Outcomes

1. **Endophytic Penicillium species**: Studies have shown that extracts from Penicillium endophytes can significantly inhibit α-glucosidase and α-amylase activities, indicating their potential in managing postprandial hyperglycemia.
2. **Colletotrichum gloeosporioides**: This endophytic fungus, isolated from medicinal plants, has produced secondary metabolites that enhance insulin secretion and improve glucose uptake in muscle cells.
3. **Fusarium species**: Extracts from Fusarium endophytes have demonstrated both antioxidant and anti-inflammatory properties, which contribute to their anti-diabetic effects by protecting pancreatic cells and improving insulin sensitivity (Boruah t al.,2024).
4. **Pestalotiopsis species**: Bioactive compounds isolated from Pestalotiopsis have shown promising results in animal models of diabetes, including improved glucose tolerance and enhanced insulin sensitivity.

Potential Applications

1. **Pharmaceuticals**: The development of novel anti-diabetic drugs based on bioactive compounds from endophytic fungi could provide more effective and safer alternatives to existing treatments.
2. **Functional Foods**: Incorporating these bioactive compounds into functional foods could offer dietary interventions for the management of diabetes.
3. **Nutraceuticals**: Formulating dietary supplements containing extracts from endophytic fungi could help in the prevention and management of diabetes.
4. **Biotechnological Applications**: Using endophytic fungi in biotechnological processes to produce large quantities of anti-diabetic compounds for pharmaceutical and nutraceutical use.

Bioprospecting Strategies

Methods and Approaches

1. **Plant Selection**: Choosing a diverse range of plants, particularly those known for their medicinal properties, as sources for isolating endophytic fungi.
2. **Isolation and Cultivation**: Using surface sterilization techniques to isolate endophytic fungi from plant tissues, followed by cultivation under controlled conditions to promote the production of bioactive compounds.

3. **Screening for Bioactivity**: Employing various bioassays to screen fungal extracts for anti-diabetic activity, including enzyme inhibition assays, cell-based assays, and animal models.
4. **Metabolite Profiling**: Using advanced techniques like HPLC, MS, and NMR to identify and characterize the bioactive compounds produced by endophytic fungi.
5. **Genetic and Genomic Approaches**: Applying molecular techniques to understand the genetic basis of bioactive compound production and to enhance the biosynthetic pathways involved.

Challenges and Limitations

1. **Isolation and Cultivation Difficulties**: Some endophytic fungi are difficult to isolate and cultivate, which can limit the discovery of bioactive compounds.
2. **Variability in Bioactive Compound Production**: The production of bioactive compounds can vary significantly depending on the fungal strain, the host plant, and environmental conditions.
3. **Complexity of Metabolite Profiles**: The complex mixture of metabolites produced by endophytic fungi can make it challenging to identify and isolate individual bioactive compounds.
4. **Regulatory and Ethical Issues**: Bioprospecting can raise ethical and regulatory concerns, particularly related to the conservation of biodiversity and the rights of indigenous communities.

Recent Advances

1. **Metagenomics and Metabolomics**: Advances in metagenomics and metabolomics have facilitated the identification of bioactive compounds and their biosynthetic pathways, even from uncultivable endophytic fungi (Jagadesh et al.,2024).
2. **CRISPR and Genetic Engineering**: The application of CRISPR and other genetic engineering techniques has enabled the manipulation of fungal genomes to enhance the production of desired bioactive compounds.
3. **Synergistic Approaches**: Combining traditional bioprospecting with modern techniques, such as high-throughput screening and bioinformatics, has improved the efficiency and success rate of discovering new bioactive compounds.
4. **Endophyte-Host Interaction Studies**: Understanding the interactions between endophytic fungi and their host plants at the molecular level has provided insights into the factors that influence bioactive compound production, leading to more targeted bioprospecting efforts.

Conclusion

The endophytic fungi thus can be considered an outstanding and untapped resource for the discovery of bioactive substances having huge therapeutic potential in respect of antioxidant as well as anti-diabetic activity. The deep relationships which they enter with their host plants have served to drive the evolution of an extremely diverse array of secondary metabolites, which impart a great many advantages to both the fungi interspersed and the host plant (Andreopoulos et al.,2024). All these bioactive compounds, such as alkaloids, terpenoids, steroids, phenolic compounds, and peptides, have been demonstrated to exhibit a wide range of pharmacological activities useful for medicinal purposes. The mechanisms of action, on the other hand, could be related to inhibition of carbohydrate-digesting enzymes, enhancement of insulin secretion, improvement of sensitivity toward insulin, and provision of antioxidant as well as anti-inflammatory protection, which adds more emphasis on the role of these biocompounds in the management and probably prevention of the consequences of diabetes and oxidative stress-related conditions. However, the way from bioprospecting to practice includes problems such as isolation and cultivation, variability in the production of metabolites, and complexity in compound identification and characterization. Regardless, rapid developments in biotechnological tools such as metagenomics, metabolomics, and genetic manipulation are gradually helping to overcome these bottlenecks, which facilitate efficient and effective exploration and utilization of endophytic fungi. As research further uncovers the intimacy of the endophyte-host relationship and hones bioprospecting to focus solely on the 'best of the best,' the possibilities also improve in the race to novel bioactive compounds and advanced therapeutics. Integration of traditional knowledge with cutting-edge scientific techniques is likely to not just expand our pharmacopeia, but it may expand our worldview regarding the importance of conservation of biodiversity and the ecological and cultural context within which these invaluable organisms thrive. Finally, bioprospecting endophytic fungi for their bioactive compounds holds considerable promise for future drug discovery and development, while still unlocking new opportunities in diabetes treatment, oxidative stress, and beyond, in sustainable ways that encourage ethical engagement with natural product research.

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