Research Article

Endophyte - Based Drug Delivery Systems: A Novel Approach To Natural Product - Based Therapeutics

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ABSTRACT

Endophytes, which are bacteria and fungi, are microorganisms that live within plant tissues. They produce bioactive compounds that are important for medicine and agriculture. This study examines various endophytic bacteria and fungi found in medicinal plants and their potential to produce biologically active metabolites. Bacterial species such as Pseudomonas aeruginosa, Micrococcus luteus, Streptomyces coeruleorubidus, Bacillus subtilis, and Microbacterium sp. are known to produce compounds like quinolones, diketopiperazines, lipopeptides, and vindoline. These compounds have antimicrobial, anticancer, and plant growth-promoting effects. Similarly, endophytic fungi like Fusarium solani, Aspergillus fumigatus, Penicillium chrysogenum, Taxomyces andreanae, and Colletotrichum gloeosporioides produce important anticancer compounds, including paclitaxel, podophyllotoxin, and other secondary metabolites. Various extraction and analytical techniques are used to isolate and characterize these compounds. These methods include solvent extraction, liquid-liquid extraction, chromatography (TLC, HPLC), and spectroscopic techniques (GC-MS, NMR). The findings indicate that endophytes are cost-effective, sustainable, and efficient sources of new bioactive compounds. They have significant potential in drug discovery, especially for developing antimicrobial and anticancer agents, as well as in agriculture.

Keywords: Endophyte - Based Drug Delivery System, Anti- Microbial, Anti- cancer, GC - MS, HPLC, NMR.

INTRODUCTION

Endopytes are microorganisms, mainly fungi and bacteria, that live inside plant tissues without causing harm. Studying endophyte - based drug delivery systems is important because they offer a rich and sustainable source of bioactive compounds with potential health benefits. Many endophytes produce secondary metabolites similar to those of the host plants, which can have antimicrobial, anticancer, and anti-inflammatory effects. This study arises from several factors. Traditional drug sources are becoming limited, and resistance to existing drugs is increasing, especially for infectious diseases and cancer. Endophytes provide a new and eco-friendly option for discovering new drugs. They can be modified or used to improve targeted drug delivery, making treatments more effective while reducing side effects. They also allow for cheaper and larger-scale production of pharmaceuticals compared to synthetic methods. [1,2,5,48]

Additionally, endophyte-based systems can support sustainable medicine by lowering reliance on overharvested medicinal plants. Their ability to produce various powerful compounds make them a promising area for future research in drug development and delivery. [1,2,5,48]

Overall, studying endophyte - based drug delivery systems is crucial for advancing innovative, effective, and sustainable therapeutic strategies. They represent a source of new natural products, opening up new avenues in drug discovery. Microorganisms associated with plants that inhabit the internal tissues of all plant species are becoming key targets for exploring new chemical entities. The discovery of the well-known anticancer drug paclitaxel accelerated research on endophytic biology and led to promising drug candidates with antimicrobial, immunosuppressant, antioxidant, and ant neurodegenerative functions. [1,2,5,48]

Currently, we must recognize the influence of natural products in clinical applications, with the pharmaceutical industry using high-throughput methods to screen plant secondary metabolites as potential starting points for new medications. Plant-associated endophytic bacteria live in various tissues, helping with plant growth and defending against disease-causing agents. These bacteria, which exist symbiotically in plant tissues without causing harm, have become a rich source of bioactive compounds with therapeutic potential. [1,2,5,48]

Plant-microorganism relationships have existed for over 400 million years. While plants appear to be single organisms, each is actually a complex community engaged in various interactions. Endophytes are the hidden world within the organs or tissues of their host plants. Among endophytic microbial species, fungal endophytes are a diverse group that lives quietly in the intracellular or intercellular spaces of host tissues, including stems, leaves, and roots. In recent years, endophyte research has shifted from merely cataloging species to exploring

their characteristics, interactions with host plants, and isolating bioactive compounds with medical potential. [1,2,5,48]

BACTERIA

Pseudomonas aeruginosa

The plant used in this study was Lilium dividii (bulb). Pseudomonas aeruginosa belongs to the family Liliaceae. The bacteria involved in this study is identified as LD-08. This plant helps investigate antibiotic resistance and pathogenesis. The extraction method used is ethyl acetate extraction. This method yields specific compounds like quinolones. [37,40]

Micrococcus luteus

Micrococcus luteus is a bacterium that belongs to the family Micrococcus. It is classified as a Gram-positive bacterium. This study focused on the plant Datura stramonium, specifically its seeds. Micrococcus luteus plays an important role in promoting plant growth. It is useful in agriculture and biotechnology. The extraction of its bioactive compounds is done using the liquid-to-liquid extraction method. One key compound identified in this bacterium is diketopiperazine. This compound is known for its biological activity and has potential applications. [7,40]

Streptomyces coeruleorubidus

The plant Ocimum sanctum is associated with the bacteria Streptomyces coeruleorubidus (GRG4). This is a Gram-negative bacterium and belongs to the Enterobacteriaceae family. Gas chromatography and mass spectrometry were used to examine the components in this bacterium. The liquid-to-liquid extraction method makes it easier to separate compounds based on their solubility. This method is used to extract bioactive ingredients. [7,40]

Bacillus subtilis

Zea mays belongs to the Poaceae family and is associated with the bacterium Bacillus subtilis. Its antibacterial properties come from the lipopeptides produced during extraction. These substances reduce the growth of the plant pathogen Botrytis cinerea, helping to decrease corn diseases and improve crop health. [39,41]

The endophytic bacterium Microbacterium sp. is a Gram-positive microorganism isolated from the interior tissues of the medicinal plant Catharanthus roseus. It is part of the Microbacteriaceae family. Vinca alkaloids, known for their anticancer properties, are produced by this plant. The bacterium that produces the bioactive substance is called vindoline. Column chromatography, TLC, HPLC, and ESI-MS methods were used to extract and purify vindoline. It serves as a precursor to important anticancer drugs like vinblastine and vincristine. Endophytic bacteria are efficient and cost-effective sources for medicinal products. [40]

FUNGI

Aspergillus fumigatus The most common endophytic fungi are Fusarium solani, Aspergillus fumigatus, and Penicillium chrysogenum. These fungi have been found in plant tissues and are used in the treatment of cancer. Taxus brevifolia, Camptotheca acuminata, and Podophyllum hexandrum produce bioactive anticancer compounds used in chemotherapy. These include podophyllotoxin, paclitaxel, and calprotectin. Solvent extraction methods like methanol, ethanol, and ethyl acetate are applied for extraction. Purification methods include column chromatography, TLC, high-performance liquid chromatography, and mass spectrometr [1,2,5,48]

Taxomyces andreanae One species of endophytic fungus linked to the formation of taxanes is Taxomyces andreanae, which is found in Taxus brevifolia. It is related to taxanes and the production of Paclitaxel, an anticancer drug. Molecular studies have confirmed the presence of genes related to Paclitaxel biosynthesis, such as phenyl propanoyl transferase and taxonomic diene synthase (txs). Extraction and analysis techniques include chromatographic methods like TLC and HPLC, spectroscopic methods, and immunoenzymatic assays. Extensive metabolic studies have not consistently verified the synthesis of Paclitaxel. This is important for ensuring a reliable industrial source of Paclitaxel. [2,5,28]

Penicillium chrysogenum

The filamentous fungus Penicillium chrysogenum comes from plant-associated sources, including internal tissues with an endophytic origin. It produces several chaetoglobosins, penochalasin I, and penochalasin J,

which are significant bioactive compounds. High - resolution mass spectrometry (HR-MS) and 1Dand 2D NMR were used to identify these substances. Chromatographic purification methods followed solvent extraction to complete the process. The metabolites showed strong antifungal activity against plant diseases and notable cytotoxic (anticancer) properties, making them valuable for pharmaceutical research and development. [1,2,48]

Colletotrichum gloeosporioides

Colletotrichum gloeosporioides is a fungus that lives inside plant tissues, including the leaves and stems of medicinal plants. It produces bioactive secondary metabolites with anticancer properties, as shown by GC-MS analysis. Solvent extraction with ethyl acetate (EA) is used to obtain these chemicals. The metabolites demonstrate both DNA protection and harmful effects on breast cancer cell lines. They trigger apoptosis and halt the cell cycle by controlling key genes linked to cancer processes. This fungus is important for making anti-cancer drugs and for development. [1,2,48]

Isolation and Extraction

Collection of Plant Material

The study of microorganisms starts with collecting plant material. We collect plant test samples like roots, stems, leaves, fruits, bark and seeds from disease-free plants. The selected plant should not have any symptoms like fungal infection, necrosis, insect damage or mechanical injury. These symptoms can mean the plant has epiphytic microorganisms, not endophytes. We use a sterilized scalpel to collect plant material and put it in polyethylene bags right away. We label each test sample with the plant name, part of the plant, date and location. We take the test samples to the lab soon as possible to prevent contamination and loss of microbial viability. If we can't process them away we store them at 4 °C and process within 24 hours. [1,2,5,48]

Preliminary Cleaning of Plant Material

Plant materials have dirt, dust, pollen and other contaminants on the surface. So we clean them before surface sterilization. We wash the plant test samples under running tap water for 5-10 minutes to remove dirt and debris. Then we rinse them with distilled water to remove impurities and dissolved salts. This step is crucial because dirt can reduce the effectiveness of surface sterilization and cause microbial contamination. [3,10,12,13]

Preparation of Plant Segment

After cleaning we cut the plant materials into pieces using a sterile blade. Stem and root segments are usually 0.5-1cm long while leaf pieces are 5mm. For leaves we cut segments from the part, including the midrib because endophytes are often in the vascular tissues. We do this under conditions preferably in a laminar airflow chamber. The small size of segments helps sterilizing agents penetrate. Allows better contact between plant tissue and culture media. [1,2,5,48]

Surface Sterilization of Plant Segments

Surface sterilization is a step in isolating endophytic microorganisms. We eliminate microorganisms on the plant tissue surface while keeping those inside. We sterilize plant segments by immersing them in sterilizing solutions. First we immerse them in distilled water for about 60 seconds. Then we use 70% ethanol for 30-60 seconds to destroy surface microorganisms. After that we transfer them to sodium hypochlorite solution (2-4%) for 3-4 minutes to remove surface contaminants. We dip them in 70% ethanol for 30 seconds to remove sodium hypochlorite traces. Finally we rinse them three times with distilled water to remove sterilizing chemicals. [3,10,12,13]

Confirmation of Surface Sterilization

We test the rinse water for microbial contamination to confirm sterilization effectiveness. We spread 100 µL of the rinse water on nutrient agar plates (for bacteria) and potato dextrose agar plates (for fungi). We incubate them at temperatures and observe for microbial growth. If there's no growth it confirms the sterilization procedure was effective. [3,10,13,20]

Inoculation of Sterilized Plant Segments

After confirming sterilization we place plant segments on filter paper to eliminate moisture. We transfer them aseptically onto culture media plates. We place segments so that cut edges touch the media surface as endophytic microorganisms emerge from internal tissues through cut surfaces. [3,10,13,20]
We use media types depending on the study objective: [1,2,5]

- Potato Dextrose Agar (PDA) for fungi
- Malt Extract Agar (MEA) for fungi
- Savoured Dextrose Agar (SDA) for fungi
- Nutrient Agar (NA) for bacteria

To prevent bacterial contamination during fungal isolation we add antibiotics like streptomycin to the medium. [3,10,12,13]

Incubation of Culture Plates

We seal inoculated plates with parafilm to prevent contamination and moisture loss. We incubate them at temperatures depending on the microorganism type. For isolation plates are incubated at 25 ± 2 °C for 7-14 days. For isolation plates are incubated at 30-37 °C for 24-72 hour. [3,10,12,13]
We observe plates daily for colonies emerging from plant tissues. We note each colony carefully based on shape, colour, texture and growth pattern. [1,2,5]

Isolation and Purification of Microbial Colonies [3,10,12,13]

When microbial growth appears we isolate colonies using an inoculating loop or needle. We transfer each colony to an agar plate to obtain a pure culture. We repeat sub-culturing until a single pure colony is produced. This step is essential because mixed cultures can give results. For isolates we perform microscopic examination using lactophenol cotton blue staining to observe fungal hyphae, spores and reproductive structures. [1,2,5,48]

Preservation of Pure Isolates

We preserve cultures properly for further research. bacterial isolates are maintained on nutrient agar slants while fungi isolates are maintained on PDA or MEA slants. We store slants at 4 °C. in a refrigerator. Label them with isolate number, plant source, date and microorganism type. We perform sub-culturing to ensure culture viability. [3,10,13,20]

Mass. Extraction of Endophytic Microorganisms (Optional Step) [1,2,5]

For studies on secondary metabolites, antimicrobial activity or bioactive compounds we subject pure isolates to mass cultivation. Fungal isolates are grown in potato dextrose broth or rice medium while bacterial isolates are grown in broth. We incubate cultures under shaking conditions to promote growth and metabolite production. We. Extract culture broth with suitable organic solvents like ethyl acetate. We concentrate the solvent extract using an evaporator at controlled temperature to obtain crude extracts, for biological evaluation. [2,5,7]

FORMULATION :

S. No	Name of Article	Extraction Method	Formulation Type	Uses	Reference [17,19,24,25]
1.	Development of low-cost plant probiotic formulations of functional endopytes for sustainable cultivational of coleus forskohlii.	1.Forskolin extraction 2. Ergosterol extraction	1.Wheat bran–based formulation 2.Talc-based formulation	Plantgrowth,Root yield, Forskolin enhancement, Disease control, Crop productivity	Anthati Mastana, Digeshwar Raneb, Syed G Dastagerb, C.S vivek Babua, development of lowcost plant probiotic formulations of functional endophytes for sustainable cultivation of coleus forskohlii, Elsevier Gmb H, 2 019 [1,2,5,48]

2.	Formulating bacterial endophyte involves preconditioning of cells and encapsulating them in amidated pectin beads.	Solvent extraction method	Encapsulated formulation using amidated pectin beads	Plantgrowth, Stress protection, Bacterial survival, Shelf life, Root colonization, Yield improvement	Mauricio Cruz Barreraa, Desiree Jakobs-Schoenwandtb, Martha Isabel Gómeza, Juan Serratoc, Silke Ruppeld, Anant V. Patelb, Formulating bacterial endophyte: pre-conditioning of cells and the encapsulation in amidated pectin beads, Elsevier BV, April 2020 [24]
3.	Formulating a suitable medium and optimizing it for maximizing Lasparaginae production from the endophytic fungus Fusarium sp.LCJ273.	Submerged fermentation + centrifugation (crude extraction)	Modified Czapek's Dox Broth (liquid medium) used under submerged fermentation (SmF)	produce L-asparaginase enzyme, Anti-cancer, Medical and food industry applications	V.antony Jenila and J. Joel gnanadoss, formulation of a suitable medium and its optimization for maximizing l - asparaginase production from endophytic fungi fusarium sp. LC J273,2018 [1,2,5,48]
4.	endophytic and rhizospheric Microorgams: an alternative for sustainable, organic,and regenerative bioinput Formulations for modern Agriculture	1.Microbial culture (fermentation) 2.Plant/algae extracts (general methods)	Bioinoculants & Biostimulants (solid, liquid, polymer forms)	Plant growth, nutrient uptake, disease control, eco-friendly farming	Isabela De l. Valente , joao H. C. wancura , Giovani L. Zabot and Marcio A. Mazutti, Endophytic and Rhizospheric microorganisms: an Alternative for sustainable, organic, and Regenerative Bioinput formulations for Modern Agriculture, 2025 [17,19,24,25]
5.	zinc oxide nanoparticles as potential delivery carrier: green synthesis by aspergillus niger endophytic fungus, Characterization , and in vitro/in vivo antibacterial	Green synthesis using Aspergillus niger (fungal extract)	Nano formulation (ZnO nanoparticle-based delivery system)	Antibacterial, Drug delivery, Infection treatment, Anticoagulant, Antidiabetic, Thrombolytic potential	Dalia H. Abdelkader, Walaa A. Negm and Alanood S. Almurshedi, Engy Elekhnawy, Duaa Eliwa, Basmah N. Aldosari, Zinc Oxide nanoparticles as potential delivery carrier: green synthesis by aspergillus niger endophytic fungus, characterization, and in vitro / in vivo antibacterial activity, 2022 [17]
6.	Forming New Silver Nanoparticles (Snps) Using the Fungal Endophyte Macrosporium	Fungal-based extracellular green synthesis	Nano formulation (Silver nanoparticles based formulation)	Antimicrobial, Antibacterial, Antifungal, wound healing	Dr. Savita Shrikant Deokar, Dr. Rajesh Ramesh Patil, Ms. Survanta R Takale, Mrs. Harshada H. Puranik, Mr. Pranit B. Kale, Ms. Pooja Nitin Maid, Dr. Rahul Shashikant

	Fasciculatum and Testing Their Antimicrobial Potential				Shivarkar, formulation of novel silver nanoparticles (snps) using fungal endophyte Macrosporium Fasciculatum and evaluation of their antimicrobial potential, 2024 [18]
7.	Formulating a new method for surface sterilization and creating culture media to isolate endophytic Actinomycetes from medicinal plants, along with testing its antibacterial activity	Submerged fermentation (fungal production]	Enzyme-based formulation (L-asparaginase)	Anti-cancer (leukemia treatment), Lymphoma, food industry.	Waheeda K and Shyam KV, formulation of novel surface Sterilization method and culture media for the isolation of endophytic actinomycetes from medicinal plants and its antibacterial activity, 2017 [3,10,12,13]
8.	Self-Nanoemulsifying drug delivery system (SNEDDS) formulation and molecular docking of mahogany seed extract (swietenia mahagoni) as anti-hyperglycemic	Soxhlet Extraction	Self-Nanoemulsifying Drug Delivery System (SNEDDS)	Anti-diabetic (anti-hyperglycemic)	Mushawwir Taiyeb, Hartati Hartati, Arwansyah, dahlia, Abd. Muis, A. Mu'nisa, Abdur Rahman Arif, liza md Salleh, 2024 [9,17,19]
9.	Improvement in Aqueous Solubility of cilnidipine by amorphous solid dispersion, its formulation into Interpenetrating polymer network Microparticls and Optimization by Box Behnken design	1.ASD → Melting method 2.Microparticles → Emulsion crosslinking method	Amorphous Solid Dispersion + IPN Microparticles	Improve solubility, Provide sustained drug release, Increase bioavailability.	Amit Kuhikar, Shagufta Khan , Komal Kharabe, Dilesh Singhavi, Girish Dahikar,I mprovement in aqueous solubility of cilnidipine by amorphous solid dispersion, its formulation into interpenetrating polymer network microparticles and optimization by Box-Behnken Design, 2020 [17,19,24,25]
10	Bioencapsulation of microbial inoculants: mechanisms, formulation	1.Extrusion method 2.Emulsification method 3.Spray drying	Bioencapsulated microbial formulation (polymer-based microcapsules)	Protect microbes from environment, Increase stability & shelflife,Controlled	Blanca Rojas - Sa nchez, Paulina Guzmán - Guzmán, Luzmaria R. Morales-Cedeño, MA. Delcarmen Orozco-

	types and application techniques	4.Gelation techniques		release in soil, Improve plant growth	mosqueda, blanca C. Saucedo Martinez, Juan M. Sanchez Yanez 1 Ayomide Emmanuel fadiji, Olubukola oluranti Babalola, Bernard R. Glick, andGustavo Santoyo, bioencapsulation of microbial inoculants: mechanisms, formulation types and application techniques, 2022 [24]
11	Pharmacological properties of biomimetic synthesized silver nanoparticles from the endophytic fungus Coniothyrium chaingmaiense, KUMBMDBT25	Ethyl acetate solvent extraction (from fungal culture)	Silver nanoparticles (AgNPs) – nanoparticle drug system	Antibacterial activity, Antifungal activity, Antioxidant activity, Anti-inflammatory activity, Cytotoxic (anticancer potential)	Manjunatha Dadayya, Megha Gowri Thippeswamy, Nagaraju Shivaiah, Thoyajakshi Ramasamudra Siddaraju, Prakash Jayaramaiah, Sowmya Hirakannavar Veeranna, Thippeswamy Basaiah, Shridhar N Mathad, Ravikumar Hemagiri Gowda, Sachin Naik, Abdulaziz Abdullah Al Kheraif & Sajith Vellappally, Pharmacological properties of biomimetic synthesized silver nanoparticles from endophytic fungus Coniothyrium chaingmaiense: Kumbmdbt-25,2025 [2,5,7]
12	recent advancesin Nanomaterials-based targeted drug delivery for preclinical cancer diagnosis and Therapeutics	Nanoemulsion Self-assembly Polymer-based synthesis Surface modification (PE Gylation, ligands)	Nanomaterial-based drug delivery system (liposomes, micelles, nanoparticles, dendrimers)	Targeted drug delivery (mainly cancer),Reduce toxicity, Improve efficacy & bioavailability, Controlled drug release	Harshita Tiwari, Nilesh rai , Swati Singh, Priyamvada Gupta, AshishVerma, Akhilesh Kumar Singh, kajal, prafull Salvi, Santhosh Kumar Singh and vibhavgautaml, recent advances in nanomaterials - based targeted drug delivery for preclinical cancer diagnosis and therapeutics, 2025 [9,17,19]

APPLICATIONS OF BACTERIA

Applications in Pharmaceuticals

Endophytic bacteria create a variety of bioactive compounds with important uses in medicine. These compounds have antioxidant, antimicrobial, anticancer, insect-repellent, and antidiabetic properties. The

substances produced by endophytes can target different pathogens that affect plants, animals, and humans. This makes them valuable for both medical and veterinary treatments. Many medicinal plants treat various diseases. Recent studies have shown that many key therapeutic compounds come from their associated endophytes. [1,2,5,48]

Endophytic bacteria from medicinal plants can produce polyketides and bioactive peptides with antimicrobial, anticancer, and antitubercular effects. Secondary metabolites produced by Acinetobacter baumannii linked to Capsicum annuum L. revealed phenolic compounds that show both pro-oxidant and antioxidant properties. Numerous discovered peptides exhibit antibacterial, antifungal, anticancer, immunosuppressive, and antimalarial characteristics. [2,5,7]

Industrial Applications

Endophytic bacteria produce extracellular hydrolytic enzymes that help them colonize host plant tissues and stimulate systemic resistance. This boosts plant defenses against pathogens. Bacterial osmoregulation and antioxidant enzymes help plants withstand salinity stress by maintaining cellular metabolism and promoting growth under tough conditions. [37,38,39,46]

Bacteria are widely used to produce industrial enzymes and bioactive secondary metabolites for making food products, dietary supplements, biofuels, pharmaceuticals, cleaning agents, and biopesticides. the endophytic bacterium pseudomonas aeruginosa L10, isolated from the roots of phragmites australis, can break down hydrocarbons and produce biosurfactants. This makes it useful in environmental cleanup and pollution control. [37,40]

Nano Biotechnology

Nanoparticles are primarily used in biotechnology. Biological synthesis methods offer more advantages than physical and chemical techniques. They reduce energy needs and hazardous waste, making the process simpler, cheaper, and more environmentally friendly. Endophytic microorganisms play a key role in reducing metallic ions to create nanoparticles. Nanosilver particles (AgNPs) generated by Pantoea ananatis show antimicrobial properties at low concentrations against candida albicans and bacillus cereus. at higher concentrations, they effectively combat. Multidrug-resistant strains of enterococcus faecium, Escherichia coli, streptococcus pneumoniae, and staphylococcus aureus. Similarly, AgNPs produced by endophytic Streptomyces species display antimicrobial, antioxidant, and larvicidal properties. These Streptomyces species also create copper (Cu) and copper oxide (CuO) nanoparticles with antibacterial and antifungal effects. [9,17,19]

Table No: 2 Recent examples of bacterial endophyte-mediated biosynthesis of nanoparticles and their activities.

Nanoparticles	Plant	Bacterial Endhopytes	Applications
Cu	Convolvulus arvensis	Streptomyces capillispiralis	Antibacterial and antifungal [1,2,5]
Ag	Coriandrum sativum	Bacillus siamensis C1	Antibacterial
MgO	Ocimum sanctum	Streptomyces coelicolor	Active against multidrug-resistant microbes [1,2,5]
Au	Annona squamosa	Pseudomonas veronii	Antibacterial

APPLICATIONS OF FUNGI

Biological Roles Of Endhopytic Fungi

endophytic fungi produce a wide variety of bioactive secondary metabolites with standard chemical structures and biological activities. These natural compounds are valuable in industry, agriculture, and medicine. The

metabolites synthesized by endophytic fungi can be used to develop pharmaceuticals, biopesticides, biofertilizers, enzymes, and other industrial products. [1,2,5,48]

Antiviral Activity

Endophytic fungi create several antiviral compounds that stop the growth and replication of viruses. These natural substances are emerging as promising candidates for new antiviral drugs. cytonic acids A and B, isolated from endophytic fungi, are novel human cytomegalovirus (HCMV) protease inhibitors found through mass spectrometry and NMR spectroscopy. These compounds effectively reduce viral growth and show strong antiviral potential. Metabolites produced by endophytic fungi associated with desert plants have activity against HIV-1 replication, suggesting their promise as sources of new antiviral agents. [1,2,5,48]

Anticancer Activity

Endophytic fungi are an important source of anticancer compounds with various mechanisms of action. One significant discovery is paclitaxel (Taxol) and its derivatives. They prevent cancer cell division by stopping tubulin depolymerization and halting mitosis. Paclitaxel is one of the world's leading anticancer drugs and treats various human cancers. The endophytic fungus Taxomyces andreanae offers an alternative method for producing paclitaxel through fermentation. Another key anticancer metabolite is torreyanic acid, a toxic quinone dimer produced by Pestalotiopsis microspora, first isolated from Torreya taxifolia (Florida torreya). The endophytic fungus Hypocrea lixii produces cajanol, another anticancer compound from Cajanus cajan. Additionally, Muscodor fragilis is the first documented endophytic fungus that can produce bioactive metabolites like podophyllotoxin and kaempferol, both known for their anticancer effects. Guanacastane diterpenoids, isolated from Cercospora sp., also show promising anticancer activity. [1,2,5,48]

Antidiabetic Activity

A non- peptidic fungal metabolite, L-783, isolated from the endophytic fungus pseudomassaria sp,. found near Kinshasa in an African rainforest, has demonstrated significant antidiabetic activity. This discovery showcases the potential of natural products as valuable sources for new medicines. Studies on endophytic fungi from Salvadora oleoides (Salvadoraceae) showed antidiabetic and hypolipidemic effects In glucose-loaded, fasting, and alloxan-induced diabetic wistar albino rats. Endophytes like Aspergillus spp., Phoma spp., and other unidentified fungal species significantly reduced blood glucose levels, indicating their promise as new antidiabetic agents. Furthermore, endophytic actinomycetes from the medicinal plants leucas ciliata and rauwolfia densiflora produce α-amylase inhibitors that slow the breakdown of complex carbohydrates into glucose and delay glucose absorption in the intestine. This mechanism helps control post-meal blood sugar levels, making these endophytic microorganisms strong candidates for developing new antidiabetic therapies. [1,2,5,48]

Immunosuppressive Activity

Subglutinol A and Subglutinol B, produced by Fusarium subglutinans from Tripterygium wilfordii, have strong immunosuppressive activity. They are used to lower allograft rejection in organ transplant patients and may help treat autoimmune diseases like rheumatoid arthritis and insulin-dependent diabetes. [1,2,48]

- **Pestaloside, pestalopyrone, and hydroxylpestalopyrone,** isolated from Pestalotiopsis• microspore, show significant phytotoxic activity and may serve as natural herbicides in agriculture. [1,2,5]
- **Pseudomycins** are antifungal peptide antibiotics that work against the human pathogen• Candida albicans. They contain unique amino acids like L-hydroxyaspartic acid, Lhydroxyaspartic L-chlorothreonine, and both D- and L-diaminobutyric acid, making them strong candidates for antifungal drug development. [1,2,48]
- **Ambuic acid,** a cyclohexenone derivative from Pestalotiopsis microspora, shows broadspectrum antimicrobial activity against several human pathogens and has potential• therapeutic uses. [1,2,48]
- **Munumbicins,** produced by Streptomyces species, exhibit strong antibacterial effects• against Gram - positive and Gram-negative bacteria. Additionally, Munumbicins E-4 and E5 show potent antimalarial activity and are roughly twice as effective as chloroquine [1,2,48]

Table No: 3 Bioactive Compounds and Biological Activities of Endophytic Fungi Isolated from Various Host Plants

Host Plant	Biology Activity	Endophytic fungi	Chemical Compound

Plumeria	Antimicrobial	Phomopsis sp.	Terpenoid
Taxus brevifolia	Anticancer	Taxomyces andreanae	Diterpenoid
Ginkgo biloba L.	Antimicrobial	Xylaria sp.YX-28 7-amino-4-	methylcoumarin
Piptadenia adiantoides	Anti-parasitical Properties	Cochliobolus sp.(UFMGCB-555)	cochlioquinone A, and isocochlioquinone A. [1,2,5]
Scapania Ciliata(Liverwort)	Immunosuppresive activity	Aspergillus sydowii	Sydoxanthone A,B [1,2,5]
Melia azedarach	Antimicrobial	Penicillium janthinellum	Citrinin (Polyketide)

Results and Discussion

1. **Endophytic microorganisms were identified as important sources of bioactive compounds.** The reviewed information indicates that both endophytic bacteria and fungi can inhabit the internal tissues of plants without causing apparent harm and can produce secondary metabolites with pharmaceutical and agricultural significance.
2. **Endophytic bacteria demonstrated diverse therapeutic potential.** Bacterial endophytes such as Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, and Microbacterium sp. were associated with antimicrobial, plant-growth-promoting, and anticancer-related activities. In particular, Microbacterium sp. associated with Catharanthus roseus was reported in relation to vindoline production.
3. **Endophytic fungi showed considerable pharmaceutical importance.** Penicillium chrysogenum, Colletotrichum gloeosporioides, Aspergillus fumigatus, and Taxomyces andreanae were described as sources of biologically active metabolites. These metabolites demonstrated antimicrobial, antifungal, cytotoxic, and anticancer potential.
4. **The isolation procedure emphasized effective surface sterilization.** Sequential treatment using distilled water, 70% ethanol, sodium hypochlorite, followed by repeated distilled-water washing was described to remove surface microorganisms while retaining internal endophytes. Absence of microbial growth from the final rinse was used as confirmation of successful sterilization.
5. **Different culture media supported the isolation of different endophytic microorganisms.** PDA, MEA and SDA were used for fungal isolation, whereas nutrient agar was used for bacterial isolation. Incubation conditions were selected according to the microbial group, and colonies emerging from the cut surfaces were considered for further isolation.
6. **Purification and preservation were essential for subsequent investigations.** Individual microbial colonies were repeatedly subcultured to obtain pure cultures. Microscopic examination was used for fungal characterization, while bacterial and fungal isolates were preserved on appropriate agar slants under refrigerated conditions for further research.
7. **Extraction and formulation approaches varied according to the desired bioactive product.** Solvent extraction, submerged fermentation, Soxhlet extraction, green synthesis, encapsulation, nanoformulation, and bioencapsulation were among the approaches described. These methods demonstrate the versatility of endophytes in developing pharmaceutical and agricultural formulations.
8. **Endophyte-derived formulations showed potential for controlled delivery and improved stability.** Formulations such as amidated pectin beads, silver and zinc oxide nanoparticles, self-nanoemulsifying systems, and polymer-based microcapsules were associated with improved microbial survival, stability, controlled release, drug delivery, and enhanced therapeutic or agricultural applications.
9. **The reviewed endophytes exhibited broad biological activities.** Reported activities included antimicrobial, antiviral, anticancer, antidiabetic, antioxidant, immunosuppressive, and antimalarial effects. Compounds such as paclitaxel, pseudomycins, ambuic acid, munumbicins, and L-783 illustrate the pharmaceutical potential of endophytic metabolites.
10. **Overall, entophytic microorganisms represent promising and sustainable sources for drug discovery and formulation development.** Their ability to produce diverse secondary metabolites and participate in

nanoparticle synthesis, bioencapsulation, and other delivery systems indicates considerable potential for developing innovative therapeutic products. However, the reported findings also highlight the need for further isolation, characterization, standardization, and validation before these endophyte-derived products can be reliably translated into pharmaceutical applications.

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