RESEARCH PAPER

Bioactive Potentiality of Secondary Metabolites of Endophytic Bacteria Isolated from *Ophiorrhiza mungos*

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ABSTRACT
Endophytes are microorganisms that reside within plant tissues, representing a promising and largely untapped source of novel bioactive compounds. The major classes of compounds found in endophytes includes alkaloids, anthraquinones, terpenoids, flavonoids, and polyphenols. Two endophytic bacterial isolates were studied to identify morphologically and biochemically, according to established protocols and further confirmed by 16S rDNA Sanger sequencing, as *Pseudomonas aeruginosa* and *Staphylococcus pasteuri*. Phylogenetic tree analysis of those identified isolates shared sequence similarities and after GenBank submission, accession numbers for the nucleotide sequences were found to be ON721386 and OQ834461 respectively. Bioactive secondary metabolite production with medicinal value, were identified, and their structures, gene associations, and protein-protein networks were analyzed by different computational tools and servers, which were reported earlier with their antimicrobial, anti-infective, antioxidant, and anti-cancer capabilities. Finally, endophytic bacteria collected from medicinal plants can provide new leading bioactive compounds which could be an effective approach to accelerate drug innovation and development.
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1. INTRODUCTION

Plants form complex relationships with a wide range of microorganisms, many of which reside internally without causing apparent harm. Of these numerous groups of microorganisms that coexist symbiotically with their plant host, endophytes are the most closely and intimately related (Bamisile et al., 2018). The term 'endophyte' was first defined by De Bary in 1866 as 'any organism that grows within plant tissues' (Xia et al., 2022). The endophytes include bacteria (Ryan et al., 2008), fungi (Rodriguez et al., 2009), archaeal taxa (Müller et al., 2015), and even eukaryotic unicellular organisms like algae and amoebae (Tejesvi et al., 2008). The endophytes are ubiquitously found in all plant parts, including roots, stems, leaves, and seeds. It is assumed that nearly every plant hosts at least one form of endophyte (Strobel and Daisy, 2003). Despite their prevalence, the functional roles of many endophytes remain incompletely understood. They may remain latent or develop mutualistic or antagonistic interactions depending on the host's physiological condition or environmental triggers (Sieber, 2007). To successfully colonize and persist in plant tissues, endophytes must overcome plant defense mechanisms through various adaptive strategies (Dudeja et al., 2012).

Recent research has demonstrated that endophytes can significantly influence plant health and development. They enhance resistance to pathogens through the production of secondary metabolites and can modulate plant defense mechanisms (Landum et al., 2016). Importantly, endophytes are increasingly recognized as a rich source of bioactive secondary metabolites with pharmacological and agricultural value, including compounds with antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory properties (Sharma et al., 2021).

Despite growing interest, a comprehensive understanding of their diverse biological roles and potential remains fragmented across disciplines. This review aims to consolidate current knowledge on the origin, classification, and functional attributes of endophytes. While several reviews have summarized the diversity and sources of endophytic microorganisms, a critical analysis of their therapeutic potential is urgently needed. Much of the existing literature focuses on the isolation and identification of new compounds, with less attention paid to their mechanisms of action, *in vivo* efficacy, and potential for clinical translation. This review aims to fill this gap by providing a critical evaluation of the therapeutic potential of endophytic secondary metabolites.

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Endophytic bacteria from medicinal plants offer the possibility of discovering a wide range of chemicals as well as a sustainable source of natural products. It has been reported that in medicinal plants, Bacillales, Enterobacterales, and Pseudomonadales were the most prevalent orders, accounting for 72.62% of the total, and *Bacillus*, *Pantoea*, and *Pseudomonas* were the most prevalent genera, accounting for 58.92%. *Bacillus*, *Pseudomonas*, and *Paenibacillus* can influence the growth, stress resistance, and metabolism of medicinal plants, and Streptomyces has been observed to promote plant growth and development. Surfactin, Iturin, Fengycin, Munumbicins, and many more bioactive compounds with anti-infective, antimicrobial, anticancer, and anti-inflammatory bioactive compounds have been purified from various medicinal plants throughout the world.

Endophytes are divided into two groups according to their biology, genetics, and mode of host-to-host transmission. These groups are systemic or true endophytes and non-systemic or transient type of endophytes. Systemic endophytes are organisms that live inside a plant, work in symbiotic relationships with their hosts, and never exhibit outward signs of illness. Under normal circumstances, organisms that reside in plant tissues for at least a portion of their life cycles do not appear to cause any disease symptoms in plants, but when the host plant is under stress or has few resources, they become pathogenic and are known as transient endophytes (Wani et al., 2015). While both types of endophytes reside within plant tissues, their relationship with the host and their stability differ considerably. True endophytes are generally considered symbiotic and beneficial, while transient endophytes can be opportunistic and potentially pathogenic (Higgins et al., 2014). These endophytes share the host's genetic and metabolic composition due to co-evolutionary selection, and they are immune to the host's defensive mechanisms and/or metabolites (Christensen et al., 2008).

Every plant has a vast population of endophytes living inside its tissues. Endophyte diversity is influenced by the host plant's genotype, tissues, growth stage (age), and health status (Fazeli, 2024). Plants host a variety of endophytes, including endophytic bacteria, fungi, and actinomycetes. The following table provides the sources of endophytic bacteria. (Table 1).

Table 1: Sources of endophytic bacteria, fungi, and actinomycetes with their host plant, habitat, and biological activity.

Endophyte	Host plant	Habitat	Activity	References
Endophytic Bacteria				
B. thuringiensis	*Physalis alkekengi L.*	Southern Europe, South and Northeast Asia	Antibacterial	(Younis et al., 2022)
Amycolatopsis tolypophora	*Stachys lavandulifolia Vahl.*	Iran, Turkey, Iraq, Caucasia, and central Asia	Antibacterial	(Wicklow et al., 2005)
Bacillus subtilis	*Allamanda cathartica L*	Brazil	Antifungal	(Younis et al., 2022)
B. licheniformis	*Moringa peregrina* (*Forssk*.)	Arabian Peninsula	Antifungal and Antibacterial	(Aljuraifani et al., 2019)
Paenibacillus	*Pachycereus pringlei (cardon cactus)*	Northwestern Mexico	Antibacterial and Antifungal	(Afzal et al., 2019)
Bacillus pumilus	*Prosopis strombulifera*	Northern and central zones of the Argentine Republic.	_	(Sgroy et al., 2009)
B. mojavensis	*Glycyrrhiza uralensis*	Southern Europe and parts of Asia	Antifungal	(Younis et al., 2022)

Bioactive compounds

Due to their exceptional metabolic adaptability, endophytic bacteria can produce a wide range of secondary metabolites with diverse structural characteristics. Terpenoids, polyketides, alkaloids, phenolics, and quinones are examples of these bioactive substances (Xu et al., 2021). These metabolites are well-known for their cytotoxic, anti-inflammatory, and antibacterial activities. Some endophytes are known to produce hybrid terpenoids with synergistic antibacterial activity, including meroterpenoids (Amirzakariya and Shakeri, 2022). Recent insights into the metabolic diversity of endophytes have expanded our understanding of endophytes as precursors of valuable metabolites involved in biotransformation, disease resistance, and pharmaceutical potential. It was shown that endophytic fungi from *Siraitia grosvenorii* could change mogroside precursors into the valuable sweetener siamenoside I, indicating their potential in low-cost metabolite production (Liu et al., 2024). A list of endophyte-derived compounds is given in Table 2.

Table 2L Bioactive compounds synthesized by endophytes.

Chemical Class	Bioactive Compound	Therapeutic properties	Endophytes	Host plant	Activity (IC_{50} Value)	References
Alkaloids	Chaetoglobosin A	Anticancer, [tested cell line-Colon cancer cell lines (HCT116)]	*Chaetomium globosum*	*Ginkgo biloba*	3–8 µM	(Gupta et al., 2023)
	Chrysogenamide A	Neuroprotective	*Penicillium chrysogenum*	*Cistanche deserticola*	1×10^{-4} µM	(Tiwari and Bae, 2022)
	Camptothecin	Possible anticancer drug that inhibits DNA topoisomerase I	*Bacillus subtilis*	*Pyrenacantha angustifolia*	NA	(Soujanya et al., 2017)
	Cytochalasin D	Anticancer	*Xylaria arbuscula* QYF	*Kandelia candel*	3.61 ± 1.60 µM	(Tan et al., 2024)
	Pyrrocidine A	Anticancer (tested on the human ovarian cancer cell line A2780)	*Cylindrocarpon* spp.	*Sapium ellipticum*	1.7 µM	(Kamdem et al., 2018)
	Piperine	Anti-inflammatory, antimicrobial	*Periconia* sp.	*Piper longum*	NA	(Verma et al., 2011)
	Quinine	Antiprotozoal	*Colletotrichum* spp.	*Cinchona ledgeriana*	NA	(Cruz et al., 2020)
Asteriquinone	Ochrindole F	Anticancer	*Aspergillus* sp. GZWMJZ−258	*Garcinia multiflora*	1.8 ± 0.1 µM	(Wang et al., 2023)
	Petromurins D				1.3 ± 0.1 µM	
	Kumbicins C				1.2 ± 0.1 µM	
Anthraquinone derivative	Emodin	Hepatoprotective, anti-inflammatory, antimicrobial, antioxidant	*Epicoccum nigrum*	*Hypericum perforatum*	NA	(Rutkowska et al., 2023)
	Hypericin	Antidepressant, anti-inflammatory, anticancer, and immunostimulant	*Epicoccum nigrum*	*Hypericum perforatum*	NA	(Vigneshwari and Va, 2019)
Benzyl ethers	Methoxymethylphenol	Antioxidant	*Chaetomium globosum,*	*Passiflora incarnata*	210–324 µg/mL	(da Silva et al., 2020)
Benzamide derivative	Fusarithioamide B	Cytotoxic	*Fusarium sp.*	*Anvillea garcinii* (Burm.f.)	0.09 µM	(Hridoy et al., 2022)
Benzoic acid	Terreic acid	Antioxidant	*Pseudocercospora* sp. *ESL 02*	*Elaeocarpus sylvestris*	176.29 mM	(Gupta et al., 2023)
Coumarin	Mellein	Monoamine oxidase inhibitor (Neurodegenera	*Colletotrichum gloeosporioides*	*Uncaria rhynchophylla*	8.93 ± 0.34 µg/mL	(Wei et al., 2016)

		tive)				
	4-hydroxy-mellein	Antifungal, acetylcholinesterase inhibitor, anti-hyperglycemic	*Penicillium* sp.	*Alibertia macrophylla*		

Compared to synthetic analogs, several endophyte-derived compounds offer multi-targeted mechanisms. For instance, camptothecin from *Neurospora* sp. mirrors the mechanism of synthetic topoisomerase I inhibitors, yet its endophytic origin allows potential for cost-effective bioproduction. Likewise, endophytic production of paclitaxel has opened opportunities for taxane biosynthesis without reliance on *Taxus* spp. However, yields are often low, and biotechnological enhancement through metabolic engineering or heterologous expression systems is required for industrial-scale production. Despite the vast diversity of endophytic microbes, the discovery of truly novel bioactive compounds remains a significant challenge. A major hurdle is the high rate of rediscovery of known compounds, which can be addressed by developing more targeted screening approaches. Furthermore, a large proportion of endophytic microbes are not culturable under standard laboratory conditions, representing a vast untapped reservoir of potential new drugs. New cultivation techniques, combined with genome mining and metabolic engineering, offer exciting opportunities to unlock this hidden chemical diversity.

Anticancer activity

Endophytic microbes are a rich and well-documented source of bioactive secondary metabolites, with anticancer compounds being among the most significant discoveries. These microorganisms synthesize a diverse group of molecules that can induce apoptosis, inhibit cell proliferation, and disrupt cancer cell signaling pathways. The anticancer mechanisms exhibited by endophytic compounds differ markedly from those of conventional chemotherapeutic agents. For instance, paclitaxel does not interfere with DNA or RNA synthesis in cancer cells, nor does it induce direct DNA damage. Instead, its primary mode of action involves promoting the polymerization and assembly of tubulin, thereby inhibiting spindle formation and resulting in cell cycle arrest (Yang and Horwitz, 2017). Furthermore, paclitaxel suppresses regulatory cells and tumor-associated macrophages, thereby enhancing anti-tumor immune responses.

MATERIALS AND METHODS

Sample collection

Fresh and healthy medicinal plants were collected from the Thodupuzha region of Kerala,India and kept into sterile zipper bags and then labeled and sealed. The samples were then immediately transferred to the lab maintaining a cold chain and stored in a 4°C refrigerator for further processing.

Isolation of endophytic bacteria

The total procedure was performed according to Ferreira et al., 2008,were cleaned under running tap water to remove debris and then air dried .Surface sterilization was carried out by rinsing them with Tween-20 for 10 minutes, followed by further washing with dH_2O at least 7 times. After that, samples were dipped into 70% alcohol for 30 seconds and then washed with dH_2O. Twenty (20.0) ml of 0.2% Hg_2Cl_2 solution was added to the samples in a beaker, which was placed on a shaker at 240 rpm for 5 minutes at 27°C. Then, the samples were washed again with dH_2O at least 7 times. The final samples rinsed water was used as a control and spread onto nutrient agar plates which contained (g/L)—peptone 5.00, beef extract 2.00, yeast extract 3.00, NaCl 5.00 and agar 18.00, where the pH was adjusted to 7.0. For the isolation of endophytic bacteria, samples were further treated in sterile phosphate-buffered saline (PBS) [39] containing (g/L) NaCl 8.00, KCl 0.20, Na_2HPO_4 1.44 and KH_2PO_4 0.24, where the pH was adjusted to 7.4 and maintained at 28°C under 50 rpm agitation. All plates, including the control, were incubated at 37°C for 5 days, and the number of CFUs was determined to estimate bacterial population density according to Addisu and Kiros, 2016. Following purification, morphologically distinct colonies were identified by observing colony characteristics such as gram nature, color, and shape using a binocular biological microscope where colonies of similar morphological features were grouped into the same species . Thus, isolates were selected, cultured, purified and stored in the laboratory at -80°C in glycerol stock (50%) solution for further studies.

Phenotypic and biochemical characterization of endophytic bacterial isolates

Standard tests for morphological and biochemical analysis were performed for the identification of endophytic bacteria. They were characterized by Gram staining and biochemical tests as described in the Cowan and Steel's Manual for the identification of medical bacteria .For the activities of oxidase, catalase, citrate utilization, indole production, methyl-red (MR), Voges-Proskauer (VP), urease production, and mannitol salt fermentation, isolates were biochemically analyzed .Then, the standard protocol of Bergey's Manual of Systemic Bacteriology was followed for identification of isolates provisionally up to the species level .

Determination of antibiotic sensitivity

The susceptibility of two identified isolates to different antibacterial agents was measured *in vitro* by employing the modified Kirby-Bauer method. This method helps to determine the efficiency of a drug rapidly by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Five commercially available antibiotic discs (Himedia, India) were used for the tests

Molecular identification of bacteria

Genomic DNA was extracted and stored at -20°C. Following a standard protocol, the DNA concentration was measured by a Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) following a standard protocol. Polymerase chain reaction (PCR) was carried out for the detection of bacteria using previously published primers and targeted genes . The Basic Local Alignment Search Tool (BLAST) was used to determine primer specificity by searching for similar sequences in the microbial genome. During all experiments, positive and negative controls were carried out. The total composition of the target gene, primer sequences, cycling parameters, PCR master mixture and amplicon size (bp) were determined for PCR amplifications in a thermal cycler (NyxTechnik). Amplified PCR products were then analyzed by electrophoresis (Micro-Bio-Tech Brand) in 2% (w/v) agarose gel in 1×TAE buffer, stained with ethidium bromide (1%) and compared with marker DNA (GeneRuler 1 kb DNA Ladder), finally visualized under ultraviolet (UV) trans-illuminator (Benda company) and then photographed. Then, PCR products were purified by an ATP^{TM} Gel/PCR Fragment DNA Extraction Kit (Catalog No. ADF100/ADF300).Biochemically identified bacterial isolates were then sent for sequencing (Macrogen, South Korea). After sequencing, the results were visualized in DNA Baser software (V 5.15) and analyzed by the BLAST program in NCBI . Then, the sequences were submitted to the GenBank database. Phylogenetic tree construction and evolutionary analyses were performed for both isolates between BLAST searches of identified bacteria using MEGA 11 software. The maximum composite likelihood method was used for computing evolutionary distances.

Screening for growth-promoting parameters

Indole acetic acid (IAA) production.

The IAA production potential was calculated as per Gordon and Weber. The endophytic bacterial isolates were grown on ISP2 broth containing 0.2% L-tryptophan incubated at 37°C with shaking at 150 rpm for 5 days. Cultures were centrifuged at 12,000 rpm for 10 min. Development of a pink–red color confirms IAA production by the addition of 0.5% Salkowski reagent into 1 ml of cell free supernatant. Estimation of IAA was measured by taking the absorbance at 530 nm using a spectrophotometer, and the amount of IAA was calculated in µg/ml compared with the standard curve of IAA.

Preparation of crude extract.

Isolated endophytic bacterial crude extracts were prepared following the methods described by Deljou and Goudarzi, 2016 with some modifications [52]. Endophytic bacterial isolates were inoculated in a 125 mL Erlenmeyer flask containing 25 mL nutrient broth. A rotary incubator shaker was used for incubation at 150 rpm and 37°C for 24 hours and 48 hours. After incubation, centrifugation was performed at 12,000 rpm for 10 minutes, and the cell and supernatant were extracted with organic solvent (1:1 v/v) using ethyl acetate (EA). A rotary vacuum evaporator was used to retrieve crude extracts by evaporating the organic solvents. The dry weight of the crude extracts was measured using a digital weighing machine and dissolved in 1% dimethyl sulfoxide (DMSO).

Total phenolic content (TPC).

The total phenolic content was measured by following Folin-Ciocalteu's colorimetric method where 0.1 mL of sample and 0.5 mL of Folin-Ciocalteu were mixed with 6.0 mL of double-distilled water. After 1 min, 1.5 mL of 20% Na_2CO_3 (Merck, Germany) was added, and the total volume was made up to 10.0 mL with double-distilled water. The mixture was incubated for 2 h at 25°C. The absorbance was measured at 760 nm using a spectrophotometer against the blank solution containing all the reagents and the appropriate volume of the same solvent used for the sample. Gallic acid was used as the control indicator containing all the reaction agents except the sample.

Trypan blue dye exclusion assay

This dye exclusion assay is used to determine the number of viable and/or dead cells in a cell suspension. Trypan blue is a large negatively charged molecule. Trypan blue dye exclusion assay is based on the principle that live cells possess intact cell membranes that exclude this dye, whereas dead cells do not. In this assay, adherent or nonadherent cells are incubated with serial dilutions of test compounds for various times. After the compound treatment, cells are washed and suspended. Cell suspension is mixed with dye and then visually exam ined to determine whether cells take up or exclude dye. Viable cells will have a clear cyto plasm, whereas dead cells will have a blue cytoplasm. Number of viable and/or dead cells per unit volume is determined by light microscopy as a percentage of untreated control cells

RESULTS

Isolation of endophytic bacteria

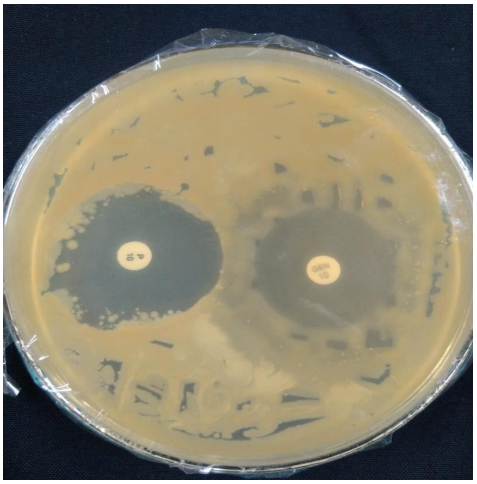
Plant parts were subjected to surface sterilization triturated with autoclaved phosphate-buffered saline (PBS) and then used to isolate endophytic bacteria. After spreading the plant extracts over solid nutrient agar (NA) media, several bacterial colonies were found, and pure colonies were selected after incubation for 48 hours at 37°C. Then, several subcultures were performed to isolate pure cultures from different types of endophytic bacteria and finally preserved in a 4°C refrigerator for further studies.

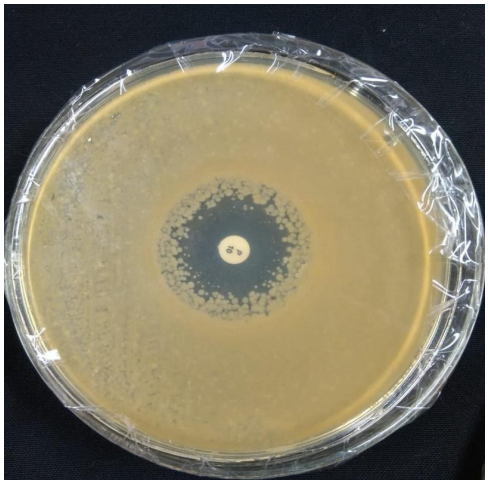
Identification of endophytic bacteria by biochemical characterization.

Pure cultures of two types of isolates were then subjected to a series of biochemical tests as described in Bergey's Manual for Determinative Bacteriology . After the Gram staining procedure, one isolate, was considered gram-negative and the others were considered gram-positive bacteria. In the indole production test, the absence of a red ring indicates a negative result. Methyl Red and Voges-Proskauer test were also showed negative results.And it shows a positive in citrate utilization test. Whereas the other isolate shows Indoel test and Citrtate utilization test a negative result and Methyl Red and VP showed positive result.

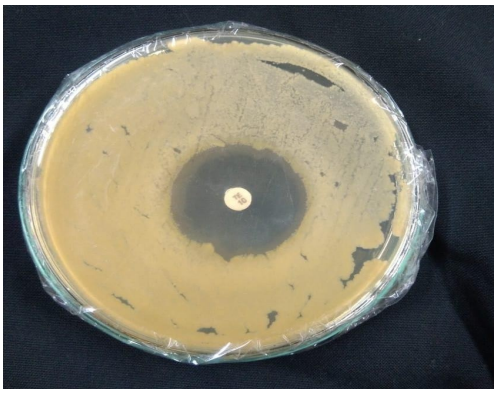
Antibiotic sensitivity test.

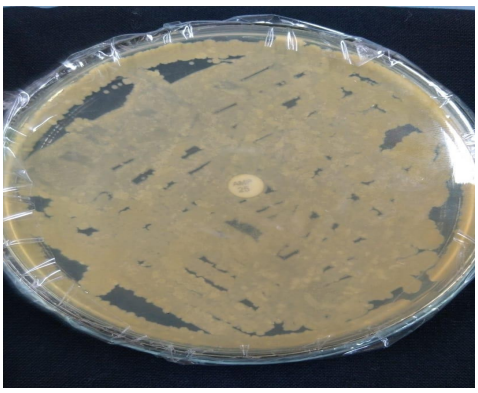
Five types of antibiotic discs (Himedia, India) were used to test the sensitivity. Except Ampicillin all other's (Tetracycline, Chloramphenicol, Penicillin, Gentamicin) show resistance to endophytic bacteria. Zone of inhibition around the disc was formed. Ampicillin is sensitive to the endophytic bacteria because, they doesn't produce zone of inhibition. It doesnt affect the growth of the bacteria present in the culture.





Identification of bacterial strains by molecular characterization.





After extraction of genomic DNA from isolates, DNA concentration and purity were measured by a Nanodrop 2000 (Thermo Scientific, USA). Extracted DNA of five bacterial isolates was then amplified by 16S rDNA primers. Gel electrophoresis was performed afterwards on a 2% agarose gel and stained with ethidium bromide. After that, the gel was visualized by a UV transilluminator (Benda Company) . After PCR amplification of bacterial isolates, they were sent for Sanger sequencing by Macrogen's sequencing service (South Korea). Purified PCR products along with their respective primers were sequenced and finally confirmed up to their species. Isolates were identified as *Pseudomonas aeruginosa* and *Staphylococcus pasteuri* respectively, with 99% identity with an e value of 0 in NCBI BLAST (Basic Local Alignment Search Tool) analysis. After analysis by the BLAST program, isolate information was submitted to GenBank by using the GenBank submission portal. Then, the accession numbers for the nucleotide sequences were obtained as ON721386 and OQ834461.The isolates were then subjected to phylogenetic tree construction according to their 16S rRNA sequences with MEGA 11 Version 5.0 Software.The neighbor-joining method was used to

interpret evolutionary history.The scale is shown in the tree, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were enumerated by applying the maximum composite likelihood method in units of the number of base substitutions per site. The analysis has 20 nucleotide sequences. Codon positions comprised with 1st+2nd+3rd+Noncoding. All cryptic positions were eliminated for each sequence pair. There were a total of 1349 positions in the final dataset. Evolutionary analyses were conducted in MEGA 11.

Viable cell suspension (1x106 cells in 0.1m1) was added to tubes containing various concentrations of the test compound and the volume was made up to lml using RPMI media. Control tubes contained only cell suspension (without additives). These tubes were incubated for 3h at 370 C. At the end of incubation cell suspension in the tubes were mixed with 0.1m1 1% trypan blue and kept for 2-3 minute and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained cells were counted separately.

CHALLENGES AND RESEARCH GAPS

Despite the vast catalog of bioactive metabolites, the translational pipeline for endophyte-based therapeutics is underdeveloped. A major challenge lies in the variability of metabolite production depending on culture conditions and host specificity. Moreover, genomic and biosynthetic pathway studies are often absent, impeding synthetic biology-based optimization. Most bioactivities are reported based on crude extract assays, which lack specificity and reproducibility. Additionally, regulatory hurdles and ecological concerns regarding wild endophyte sourcing must be addressed to ensure ethical and sustainable utilization. To accelerate the translation of endophyte research into new medicines, multifaceted technologies should be adopted.

FUTURE RESEARCH APPROACH

Future research on endophytes should focus on unraveling the molecular mechanisms underlying their diverse bioactivities, particularly through advanced omics technologies such as genomics, transcriptomics, proteomics, and metabolomics. Integrative studies can help identify novel bioactive compounds with enhanced specificity and efficacy for pharmaceutical, agricultural, and industrial applications. There is also a growing need to explore the synergistic interactions between endophytes and their host plants to better understand their role in stress tolerance, disease resistance, and metabolite biosynthesis. Moreover, sustainable biotechnological approaches, including synthetic biology and genome editing, should be employed to optimize endophyte-based production systems. Expanding the exploration of endophytes from underexplored ecosystems and rare plant species may also yield unique strains with unprecedented bioactivities. Finally, a concerted effort is needed to move the most promising endophytic compounds into clinical trials to assess their safety and efficacy in humans.

CONCLUSION

Endophytic microbes represent a vast and still largely unexplored reservoir of chemical diversity with immense therapeutic potential. They produce diverse secondary metabolites with anticancer, antimicrobial, antioxidant, and anti-inflammatory properties. In agriculture, endophytes enhance plant resilience and contribute to disease management. However, to unlock this potential, the field needs to move beyond descriptive studies and embrace a more critical and hypothesis-driven approach. Classical, culture-based methods have been foundational, yet they are inherently limited by the fact that a vast majority of microorganisms are not culturable under standard laboratory conditions. While modern molecular techniques have begun to overcome this by revealing a greater microbial diversity, they are not without their own constraints. Therefore, the future of the field depends on an integrated strategy. By combining the strengths of both classical and molecular approaches with emerging technologies like multi-omics and synthetic biology, we can overcome these limitations. This review has critically assessed the current state of knowledge, highlighting not only the successes but also the challenges and knowledge gaps. By providing a roadmap for future research, we hope to inspire a new wave of innovation in the field, ultimately leading to the development of new drugs from these fascinating microorganisms. The journey from the plant microbiome to medicine is long and challenging, but the potential rewards are immense.

DECLARATION OF COMPETING INTEREST

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

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