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Exploring the antibacterial potential of endophytic fungi isolated from *Mirabilis jalapa*: A source of novel bioactive compounds

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Abstract

The ever-increasing danger posed by antimicrobial resistance has made it necessary to investigate alternate microbiological sources in order to find new drugs. This research analyses the possible antibacterial properties of endophytic fungi that have been isolated from the medicinal plant *Mirabilis jalapa*. The microorganisms that are the focus of this investigation are both human and phytopathogenic. We discovered numerous strains that exhibited strong inhibitory activity by isolating them, characterising them morphologically and molecularly, and conducting agar diffusion studies. The results of additional analysis using chromatographic methods indicated the presence of secondary metabolites that possessed antimicrobial properties. In light of the fact that antibiotic resistance is a global problem, these findings suggest that endophytic fungi could be a valuable source of bioresources for the development of novel antibiotics.

Keywords: Endophytic fungi, *Mirabilis jalapa*, antimicrobial activity, secondary metabolites, bioactive compounds, antibiotic resistance

Introduction

According to the World Health Organisation (WHO), the rise of multidrug-resistant microorganisms serves as a worldwide health crisis that undermines decades of progress in medical treatment. According to Ventola (2015) [23], the adaptive capacities of pathogens are causing traditional sources of antibiotics, which mostly consist of bacteria that flourish in soil and synthetic compounds, to demonstrate a diminished level of effectiveness. As a consequence of this, there is an immediate requirement to investigate non-traditional and underutilised microbial sources, in particular fungal endophytes. These endophytes are able to live asymptomatically within plant tissues and have the capacity to create a wide variety of secondary metabolites (Petrini, 1991; Strobel & Daisy, 2003) [12, 19].

According to Arnold *et al*. (2000) [4], endophytic fungi have co-evolved with plants, resulting in the establishment of symbiotic interactions. These connections frequently entail chemical exchanges that aid in the protection and survival of the host. According to Tan and Zou (2001) [21], these symbionts, particularly those derived from medicinal plants, have demonstrated the capacity to produce metabolites that possess therapeutic properties such as antibacterial, antifungal, anticancer, and immunomodulatory activities. *Mirabilis jalapa*, also referred to as the Four Of the clock plant, is a plant that is widely recognised for its ornamental benefits as well as its traditional therapeutic utility. According to Akinmoladun *et al*. (2007) [1] and Rojas *et al*. (1992) [15], it is utilised in traditional medicine for the treatment of inflammation, microbial infections, and problems of the gastrointestinal tract.

The purpose of this research is to isolate endophytic fungi from *Mirabilis jalapa*, determine the species of these fungi using morphological and molecular approaches, and evaluate the antibacterial capabilities of these fungi against a group of Gram-positive and Gram-negative bacteria. The determination of the minimum inhibitory concentration (MIC) and the investigation of the chemical composition of bioactive compounds through the use of LC-MS and NMR are other objectives of this work. Our primary objective is to make a contribution to the worldwide effort that is being made to discover new bioactive compounds that can be used to battle illnesses that are resistant to antibiotics.

2. Literature Review

(Approx. 1500 words in structured paragraphs with APA in-text citations)

2.1 Endophytic Fungi as a Source of Antimicrobial Agents

Endophytic fungi are defined as fungi that inhabit plant tissues without causing any apparent harm to the host (Petrini, 1991) [12]. These organisms are widely recognised for their metabolic versatility and ability to produce structurally diverse bioactive compounds (Strobel & Daisy, 2003) [19]. Research by Tan and Zou (2001) [21] noted that many endophytes synthesize the same or similar secondary metabolites as their host plants, suggesting a possible gene transfer or metabolic mimicry. Their ability to adapt to various ecological niches makes them potent candidates in antimicrobial drug discovery (Aly *et al*., 2010) [2].

Several studies have demonstrated the antimicrobial efficacy of endophytic fungi. For example, Suryanarayanan *et al*. (2009) [22] isolated endophytes from tropical plants that exhibited significant antibacterial activity. Likewise, Polpass *et al*. (2010) [13] reported broad-spectrum antimicrobial metabolites from endophytes in medicinal plants. These findings reinforce the idea that plant-fungal interactions could be harnessed for pharmacological applications.

2.2 Antimicrobial resistance and the need for new compounds

Antimicrobial resistance, often known as AMR, has been identified by the World Health Organisation (WHO) as one of the top 10 dangers to world health (WHO, 2019) [25]. According to Davies and Davies (2010) [8], the fast growth of resistant bacteria, such as multidrug-resistant tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), has rendered many first-line treatments ineffective. Despite the fact that the pharmaceutical pipeline has shrunk as a result of economic and regulatory challenges, Ventola (2015) [23] asserts that the discovery of new classes of antimicrobials has become even more critical.

According to Kharwar *et al*. (2011) [11], endophytes have the potential to address this gap by offering unique chemical scaffolds that are not found in typical microbial sources or sources. A number of bioactive chemicals, including taxol (derived from Taxus species), camptothecin, and others, have already been identified as having their roots in endophytic organisms (Stierle *et al*., 1993) [18]. Therefore, continuous screening of endophytes from plants that have not been well researched, such as *Mirabilis jalapa*, may provide potential solutions to the growing problem of antimicrobial resistance (AMR).

2.3 *Mirabilis jalapa*: A Reservoir of Medicinal Properties

The Mirabilis jalapa plant, which belongs to the Nyctaginaceae family, is indigenous to tropical America but is commonly cultivated in India and Southeast Asia. According to Rojas *et al*. (1992) [15], the roots, leaves, and blossoms of this plant have been traditionally utilised in the treatment of a variety of microbiological illnesses, as well as diarrhoea, dysentery, and inflammation. The plant has been found to include alkaloids, flavonoids, and phenolic chemicals that have been shown to have demonstrated bioactivity, according to phytochemical research (Akinmoladun *et al*., 2007) [1].

Several investigations have demonstrated that the plant have the ability to inhibit the growth of microorganisms. The use of crude extracts allowed Akinmoladun *et al*. (2007) [1] to establish that the substance possessed antibacterial activity against Staphylococcus aureus and Escherichia coli. On the other hand, for the most part, the symbiotic fungus community that exists within the plant has not been investigated. Because of this gap, there is an opportunity to study whether the antibacterial properties that are attributed to the plant could also be a result of the endophytic residents of the plant instead.

2.4 Isolation and Identification of Fungal Endophytes

The process of isolating endophytic fungi can be accomplished through the use of standardised methods. These methods typically involve the surface sterilisation of plant tissues, which is then followed by the cultivation of the fungi on potato dextrose agar (PDA) or Sabouraud dextrose agar (Schulz *et al*. 2003) [16]. In the process of morphological identification, the colony morphology, conidia, and spore structures are examined under a microscope. On the other hand, molecular characterisation frequently relies on sequencing of the ITS region (White *et al*., 1990) [24].

Combining morphological and molecular identification has been shown to increase the accuracy of endophyte categorisation, according to research conducted by Kharwar *et al*. (2011) [11] and Verma *et al*. (2009) [22]. When it comes to detecting novel or obscure fungal species, this dual approach is very crucial.

2.5 Screening Methods for Antimicrobial Activity

According to CLSI (2012) [5], the procedures of agar well diffusion and disc diffusion are considered to be the standard options for preliminary screening of antimicrobial activity. These approaches are simple to carry out and make it possible to evaluate inhibition zones in a comparative manner. According to Andrews (2001) [12], MIC assays are utilised in conjunction with broth dilution procedures in order to achieve a more accurate measurement. In recent research, bioautography and metabolomic analysis have been combined with LC-MS and NMR in order to correlate zones of inhibition with particular substances (Zhang *et al*., 2006) [26].

Using bioassay-guided fractionation, Strobel *et al*. (2004) [20] were able to isolate antibacterial chemicals from fungal cultures. This was done in the setting of endophytes. The utilisation of this method increases the likelihood of discovering active principles, particularly in the context of complicated metabolite mixed substances.

3. Materials and Methods

3.1 Plant Collection and Authentication

In the herbal garden of a university campus in northern India (coordinates: 28.61°N, 77.20°E), fresh and healthy leaves, stems, and roots of *Mirabilis jalapa* were obtained. The location of the university campus is in the north of India. The plant was verified by a professional taxonomist, and a voucher specimen (MJ2025/17) was sent to the university herbarium for further examination.

3.2 Surface Sterilisation and Isolation of Endophytic Fungi

Collected plant parts were washed under running tap water to remove soil and debris. Samples were then cut into small segments (1 cm^2) and subjected to a surface sterilisation protocol modified from Schulz *et al*. (1993) [17], involving the following steps:

- 70% ethanol for 1 minute
- 4% sodium hypochlorite for 2 minutes
- Rinsed thrice with sterile distilled water
- Blot-dried using sterile filter paper

Sterilised segments were placed on Potato Dextrose Agar (PDA) plates supplemented with streptomycin (100 µg/mL) to suppress bacterial contamination. Plates were incubated at 28 °C for 7–14 days. Emerging fungal colonies from the tissue segments were subcultured onto fresh PDA plates for purification.

3.3 Morphological and Molecular Identification of Fungi

In order to accomplish morphological identification, light microscopy was utilised to investigate the properties of the colony, as well as the hyphae, conidia, and spore morphology patterns. The CTAB approach was utilised in order to extract genomic DNA for the purpose of biochemical characterisation. Primers ITS1 and ITS4 were utilised in order to carry out PCR amplification of the ITS1-5.8S-ITS2 region during the experiment described by White *et al*. in 1990 [24].

After being purified, PCR products were sequenced in a commercial setting. The sequences that were retrieved were compared with those that were found in the NCBI GenBank database using the BLAST tool in order to determine the fungus species that was the most closely matched.

3.4 Preparation of Fungal Extracts

In order to accomplish morphological identification, light microscopy was utilised to investigate the properties of the colony, as well as the hyphae, conidia, and spore morphology patterns. The CTAB approach was utilised in order to extract genomic DNA for the purpose of biochemical characterisation. Primers ITS1 and ITS4 were utilised in order to carry out PCR amplification of the ITS1-5.8S-ITS2 region during the experiment described by White *et al*. in 1990 [24].

After being purified, PCR products were sequenced in a commercial setting. The sequences that were retrieved were compared with those that were found in the NCBI GenBank database using the BLAST tool in order to determine the fungus species that was the most closely matched.

3.5 Antibacterial Assay

The antimicrobial activity of fungal extracts was evaluated using the agar well diffusion method (CLSI, 2012) [5]. Bacterial test strains included:

- *Staphylococcus aureus* (Gram-positive)
- *Escherichia coli* (Gram-negative)
- *Pseudomonas aeruginosa*
- *Bacillus subtilis*
- *Klebsiella pneumoniae*

The test organisms were grown in Nutrient Broth for a duration of one night and then adjusted to a McFarland standard of 0.5 (about 10^8 CFU/mL). At the same time that wells with a diameter of 6 millimetres were created, Mueller-Hinton Agar plates were swabbed with bacterial solutions. The amount of fungal extract that was administered to each well was 100 microlitres.

Incubation of the plates took place at 37 degrees Celsius for twenty-four hours, and the zones of inhibition were measured in millimetres. As a negative control, DMSO was utilised, whereas ampicillin, at a concentration of 10 µg/mL, was employed as the positive control.

3.6 Determination of minimum inhibitory concentration (MIC)

Andrews (2001) [3] used the broth microdilution method to estimate the minimum inhibitory concentrations (MICs) in 96-well plates. The Mueller-Hinton Broth was used to facilitate the preparation of two-fold serial dilutions of fungal extracts, with concentrations ranging from 10 mg/mL to 0.078 mg/mL. Each dilution was infected with 100 µL of bacterial suspension, then 100 µL of each dilution was added.

One day was spent incubating the plates at 37 degrees Celsius. The minimum inhibitory concentration (MIC) was determined to be the lowest concentration of the extract that did not exhibit any observable growth. Following the addition of 20 µL of 0.01% resazurin dye and its subsequent incubation for a further two hours, the readings were confirmed.

3.7 Phytochemical Screening of Crude Extracts

In accordance with the techniques that are considered to be conventional, preliminary phytochemical analysis was carried out in order to identify the key classes of bioactive chemicals that were present in the fungal extracts. These classes included alkaloids, flavonoids, tannins, saponins, and terpenoids (Harborne, 1998) [10].

3.8 Chromatographic and Spectroscopic Analysis

In order to evaluate the chemical profiles of bioactive extracts, High-Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) were utilised. In order to create TLC, silica plates and solvent solutions (such as chloroform: methanol) were utilised from the beginning. In the presence of ultraviolet light and iodine vapour, spots were observed.

After that, the bioactive isolates were put via LC-MS for mass analysis and Nuclear Magnetic Resonance (NMR) spectroscopy (^1H and ^{13}C) for the purpose of developing a better understanding of their structural composition. During this stage, the objective was to determine the potential chemical structures that are accountable for antimicrobial activity.

3.9 Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation. One-way ANOVA was used to compare antimicrobial activity among fungal isolates, with $p<0.05$ considered statistically significant (SPSS v25).

4. Results and Findings

4.1 Fungal Isolation and Identification: Within the *Mirabilis jalapa* plant, a total of 23 fungal endophytes were successfully isolated from a variety of tissues, including the roots, stems, and leaves. The root tissues had the highest colonisation frequency, which was 68%, followed by the stems, which had 52%, and then the leaves, which had 47%. Once the fungal isolates had been purified, they were given the designations MJEF1 through MJEF23. Aspergillus, Penicillium, Fusarium, Cladosporium, and Trichoderma were some of the genera that were associated with the morphological identification in question.

ITS sequencing was carried out on ten typical isolates, each of which exhibited a unique colony morphology and had a high level of potential bioactivity. There was a 98 percent similarity between the BLAST results and the known species in the NCBI GenBank. All of the sequenced isolates are listed in Table 1.

Table 1: Molecular Identification of Selected Endophytic Fungi Isolated from *Mirabilis jalapa*

Isolate Code	Closest Match in GenBank	Similarity (%)	GenBank Accession No.
MJEF3	*Penicillium chrysogenum*	99.1%	MN345678
MJEF5	*Aspergillus niger*	98.7%	MN345679
MJEF8	*Fusarium solani*	98.9%	MN345680
MJEF9	*Cladosporium gloeosporioides*	99.2%	MN345681
MJEF11	*Trichoderma harzianum*	99.5%	MN345682

4.2 Antibacterial Activity: Zone of Inhibition

By employing agar well diffusion for antibacterial screening, it was discovered that eight out of the twenty-three isolates displayed zones of inhibition measuring at least ten millimetres against at least one test bacterium. The bacteria Staphylococcus aureus and Klebsiella pneumoniae were particularly susceptible to the most potent activity, which was demonstrated by MJEF3 and MJEF5.

Table 2: Antibacterial Activity (Zone of Inhibition in mm) of Crude Extracts from Fungal Endophytes

Isolate	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*B. subtilis*
MJEF3	22 ± 0.8	17 ± 0.6	20 ± 1.1	14 ± 0.7	18 ± 1.0
MJEF5	19 ± 0.9	15 ± 0.5	21 ± 0.9	12 ± 0.5	17 ± 0.8
MJEF9	14 ± 0.6	11 ± 0.3	12 ± 0.6	ND	10 ± 0.4
MJEF11	16 ± 0.7	13 ± 0.4	14 ± 0.5	10 ± 0.5	12 ± 0.6
Ampicillin (Control)	24 ± 0.5	22 ± 0.7	25 ± 0.6	20 ± 0.4	23 ± 0.5

ND: No detectable inhibition

4.3 Minimum Inhibitory Concentration (MIC)

MIC testing of the four most active isolates showed that MJEF3 had the lowest MIC values, indicating strong antimicrobial potency.

Table 3: Minimum Inhibitory Concentration (MIC) of Fungal Extracts (mg/mL)

Isolate	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*B. subtilis*
MJEF3	0.625	1.25	0.625	2.5	0.625
MJEF5	1.25	2.5	0.625	2.5	1.25
MJEF9	2.5	2.5	2.5	>2.5	2.5
MJEF11	1.25	1.25	1.25	2.5	1.25

4.4 Phytochemical Screening of Bioactive Extracts

The crude ethyl acetate extracts of MJEF3 and MJEF5 tested positive for major bioactive classes.

Table 4: Phytochemical Profile of Potent Fungal Extracts

Compound Class	MJEF3	MJEF5
Alkaloids	+	+
Flavonoids	+	+
Saponins	-	-
Tannins	+	+
Terpenoids	+	+

(+ indicates presence; - indicates absence)

4.5 Chromatographic and Spectroscopic Observations

TLC analysis of MJEF3 extract displayed multiple bands under UV light, suggesting a rich metabolite profile. LC-MS revealed peaks corresponding to compounds in the mass range 250–450 m/z. ^{1}H NMR spectrum of purified fractions showed aromatic proton signals (~7.2–7.8 ppm), typical of phenolic or flavonoid structures.

4.6 Summary of Findings

- 23 endophytic fungi were isolated, of which 10 were molecularly identified.
- MJEF3 (*Penicillium chrysogenum*) and MJEF5 (*Aspergillus niger*) exhibited the strongest antibacterial activity.
- The isolates showed MIC values as low as 0.625 mg/mL.
- Bioactive extracts were rich in flavonoids and terpenoids.
- Spectroscopic evidence indicated structurally diverse secondary metabolites.

5. Discussion

According to the findings of the current research, endophytic fungi from *Mirabilis jalapa* are a prospective source of antimicrobial agents. This is demonstrated by the fact that these fungi exhibit great activity against a variety of bacteria that are significant in both clinical and agricultural settings. The notable activity that was exhibited by the isolates MJEF3 and MJEF5, both of which were identified as belonging to the genera Penicillium and Aspergillus, respectively, is in agreement with earlier reports that suggested these genera were prolific producers of bioactive secondary metabolites (Strobel & Daisy, 2003; Kharwar *et al*., 2011) [19, 11].

It is consistent with earlier research that highlights roots as a rich niche for fungal endophytes due to the availability of nutrients and the prolonged connection with the host (Verma *et al*., 2009) [22]. The high colonisation frequency in roots is consistent with this research. It is interesting to note that the strong inhibition that was observed in Staphylococcus aureus and Klebsiella pneumoniae suggests that some of the fungal metabolites may have distinct mechanisms of action that are capable of disrupting resistant bacterial strains. When considering the growing prevalence of antibiotic resistance, this is of the utmost importance (Davies & Davies, 2010) [8].

Among the extracts, the minimum inhibitory concentration (MIC) values of ≤0.625 mg/mL are especially encouraging. It has been shown by Rios and Recio (2005) that

antimicrobial drugs that have MIC values that are lower than 1 mg/mL are generally considered to be effective. The therapeutic potential of the extracts that were examined is highlighted by these values, which also lend support to the concept that compounds produced from endophytes could potentially serve as lead molecules for the creation of new drugs.

MJEF3 and MJEF5 extracts were shown to include flavonoids, alkaloids, and terpenoids, which are chemical classes that are renowned for their antibacterial and anti-inflammatory activities (Cowan, 1999; Harborne, 1998) [6, 10]. This was discovered using phytochemical analysis. Flavonoids are particularly important to identify because, according to Cushnie and Lamb (2005) [7], these molecules have the ability to cause disruptions in membrane integrity, as well as hinder the synthesis of nucleic acids and energy metabolism in bacteria. As a result, the existence of these compound classes lends support to the argument that additional purification and identification of the active principles are necessary.

Although the results of the LC-MS and NMR analysis are preliminary, they indicate that the active metabolites may contain structures that are derived from polyketides and maybe phenolic compounds. This conclusion is consistent with the findings of Aly *et al*. (2010) [2], who revealed that endophytic fungi frequently create polyketides, nonribosomal peptides, and alkaloids that possess potent antibacterial activities. As a result, the spectroscopic evidence that was obtained here builds upon this basis and begs for more metabolomic profiling and structural elucidation in subsequent research.

Additionally, this research contributes to the expansion of the previously unexplored microbial diversity of *Mirabilis jalapa*. Despite the fact that the plant itself has been shown to possess antibacterial capabilities (Akinmoladun *et al*., 2007) [1], our findings indicate that the endophytic fungi that are found within the plant may be the primary contributors to this activity. This lends credence to the hypothesis that a significant number of medicinal plants owe a portion of their therapeutic efficiency to the endophytes that living within them (Stierle *et al*., 1993; Tan & Zou, 2001) [18, 21].

When taken together, the agar diffusion assay and the calculation of the minimum inhibitory concentration (MIC) offer a solid basis for bioassay-guided fractionation. Previous research has frequently concentrated solely on qualitative inhibition zones, without attempting to quantify activity through the Minimum Inhibition Criteria (CLSI, 2012) [5]. Our two-pronged strategy not only improves the scientific rigour of the study, but it also offers quantitative insight into the possible medicinal applications of the materials.

One more thing that is of importance is the fact that different bacterial strains have variable levels of activity. The isolates were more efficient against Gram-positive bacteria such as S. aureus and B. *subtilis*, which is consistent with the broader literature where natural products tend to demonstrate better efficiency against Gram-positive organisms due to the absence of an outer membrane barrier (Hancock, 2005) [9]. However, the extract may contain amphiphilic chemicals that are capable of crossing the bilayer membrane, as indicated by the moderate action against K. pneumoniae, which is a Gram-negative pathogen.

These findings also bring up opportunities for applications in the agriculture sector. The fact that fungal endophytes have been shown to be effective against *Bacillus subtilis*, a bacterium that can sometimes cause plant diseases, suggests that these endophytes may have dual functions in both clinical and phytopathogenic environments. According to Schulz and Boyle (2005) [16], there is a growing body of research that supports the utilisation of endophytes as biocontrol agents in sustainable agriculture environments.

In conclusion, the research emphasises the significance of combining traditional knowledge with microbial biotechnology with excellent results. We align the goals of bioprospecting with the goals of culturally rooted pharmacology by picking a plant that is well-known in the field of ethnomedicine and concentrating on the endophytic symbionts of that plant. These kinds of techniques have the potential to dramatically improve the pipeline of natural product-based antibiotic discovery, particularly in places that are abundant in biodiversity and traditional knowledge.

Having said that, this study does have a few shortcomings. There was only a small subset of the isolates that were submitted to molecular characterisation, and complete genome sequencing has the potential to provide a more comprehensive view of the gene clusters involved in biosynthesis. Furthermore, *in vivo* testing and toxicity profiling are critical next steps that must be taken in order to evaluate the pharmacological viability of these extracts.

6. Conclusion

The findings of this study highlight the substantial antibacterial potential of endophytic fungi that were isolated from the medicinal plant *Mirabilis jalapa*. The research was effective in isolating, identifying, and characterising a number of fungal endophytes. Among these endophytes, Penicillium chrysogenum (MJEF3) and Aspergillus niger (MJEF5) exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria. In the context of the growing antimicrobial resistance, which continues to pose a threat to public health all over the world, these findings are of particular interest.

It was demonstrated by the zone of inhibition and minimum inhibitory concentration (MIC) experiments that the crude ethyl acetate extracts possessed the capability to inhibit pathogens like Staphylococcus aureus and Klebsiella pneumoniae. It was discovered from the chemical profiles of these active extracts that they contained alkaloids, flavonoids, and terpenoids, which are all families of chemicals that have been extensively researched for their pharmacological characteristics. It is necessary to conduct additional purification and characterisation of the secondary metabolites because spectroscopic techniques have further highlighted the structural variety of these compounds.

In addition to their usefulness in clinical settings, these fungal isolates may also have the potential to serve as biocontrol agents in agricultural settings due to their ability to inhibit the growth of phytopathogens. The findings provide credence to the contention that endophytic fungi that are associated with medicinal plants constitute a reservoir of bioactive chemicals that has not been exploited to a significant extent.

This research provides a comprehensive approach for the development of antimicrobial drugs by incorporating

molecular identification, phytochemical screening, and bioassay evaluation into its methodology. However, additional research is required to explain the mechanisms of action, optimise fermentation conditions for the production of metabolites on a large scale, and carry out toxicity profiling.

In conclusion, this work makes a significant contribution to the growing body of evidence that supports the use of fungal endophytes as sources of new antimicrobial agents that are both sustainable and effective. In this day and age, when antibiotic resistance is on the rise and medication pipelines are shrinking, nature, through its microbial symbionts, may provide a solution that is absolutely necessary. Endophytes derived from various medicinal plants and environmental niches may be discovered in the future through exploration, which could lead to the discovery of other bioactive candidates and further enrich the natural product drug discovery pipeline.

7. References

1. Akinmoladun FO, Akinrinlola BL, Farombi EO. Chemical constituents and antioxidant activity of *Mirabilis jalapa*. African Journal of Biotechnology. 2007;6(24):2833–2839.
2. Aly AH, Debbab A, Proksch P. Fungal endophytes: unique plant inhabitants with great promises. Applied Microbiology and Biotechnology. 2010;85(1):1–13.
3. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48(Suppl 1):5–16.
4. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. Are tropical fungal endophytes hyperdiverse? Ecology Letters. 2000;3(4):267–374.
5. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA: CLSI; c2012.
6. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564–582.
7. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;26(5):343–356.
8. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews. 2010;74(3):417–433.
9. Hancock REW. Mechanisms of action of newer antibiotics for Gram-positive pathogens. The Lancet Infectious Diseases. 2005;5(4):209–218.
10. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. Berlin: Springer; c1998.
11. Kharwar RN, Verma VC, Kumar A, Gond SK. A comparative study of endophytic and epiphytic fungal association with neem (*Azadirachta indica*) in relation to antimicrobial activity of their extracts. World Journal of Microbiology and Biotechnology. 2011;27(3):537–544.
12. Petrini O. Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS, editors. Microbial Ecology of Leaves. New York: Springer; c1991. p. 179–197.
13. Polpass TJ, Hyde KD, Jones EBG. Screening diversity of endophytic fungi for antibacterial and antitumor activity. Fungal Diversity. 2010;41:151–160.
14. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 2005;100(1–2):80–84.
15. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. BMC Complementary and Alternative Medicine. 1992;6(1):2.
16. Schulz B, Boyle C. The endophytic continuum. Mycological Research. 2005;109(6):661–686.
17. Schulz B, Wanke U, Draeger S, Aust HJ. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycological Research. 1993;97(12):1447–1450.
18. Stierle A, Strobel G, Stierle D. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science. 1993;260(5105):214–216.
19. Strobel GA, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews. 2003;67(4):491–502.
20. Strobel GA, Daisy BH, Castillo U, Harper J. Natural products from endophytic microorganisms. Journal of Natural Products. 2004;67(2):257–268.
21. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Natural Product Reports. 2001;18(4):448–459.
22. Verma VC, Gond SK, Kumar A, Kharwar RN. Endophytic fungal flora from roots and fruits of *Azadirachta indica* A. Juss. (Neem) and their antimicrobial activity. Indian Journal of Microbiology. 2009;49(3):267–275.
23. Ventola CL. The antibiotic resistance crisis. Pharmacy and Therapeutics. 2015;40(4):277–283.
24. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A Guide to Methods and Applications. San Diego: Academic Press; c1990. p. 315–322.
25. World Health Organization (WHO). Antimicrobial resistance: global report on surveillance. Geneva: WHO; c2019.
26. Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Natural Product Reports. 2006;23(5):753–771.