

Chapter 5

Natural Antibacterial and Antibiofilm Agents from *Heliotropium indicum*: A Strategy against Multidrug-Resistant Bacteria

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Abstract

The emergence of multidrug-resistant (MDR) bacteria has become a major global health challenge due to the increasing failure of conventional antibiotics. Medicinal plants are gaining significant attention as alternative sources of novel antimicrobial agents. *Heliotropium indicum* L., a medicinal plant belonging to the family Boraginaceae, has been traditionally used in folk medicine for the treatment of infections, wounds, and inflammatory diseases. Phytochemical studies have revealed that the plant contains several bioactive compounds including alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids. These secondary metabolites contribute to the plant's antimicrobial and therapeutic properties. Several experimental studies have demonstrated that extracts of *Heliotropium indicum* exhibit significant antibacterial activity against both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* species. In addition to antibacterial effects, the plant has shown potential antibiofilm activity by inhibiting bacterial adhesion, disrupting established biofilms, and interfering with

quorum sensing mechanisms. These activities are particularly important in combating multidrug-resistant pathogens that rely on biofilm formation for survival and persistence. The present chapter provides an overview of the phytochemical constituents, antibacterial mechanisms, and antibiofilm potential of *Heliotropium indicum*, highlighting its potential as a promising source of novel antimicrobial agents against multidrug-resistant bacteria.

Keywords: Heliotropium indicum, antibacterial activity, antibiofilm activity, multidrug-resistant bacteria, phytochemicals, medicinal plants.

1. Introduction

The rapid emergence of multidrug-resistant (MDR) bacterial pathogens has become one of the most significant threats to global public health. Overuse and misuse of antibiotics in healthcare and agriculture have accelerated the development of resistance mechanisms among pathogenic bacteria. As a result, many conventional antibiotics have become ineffective in treating infectious diseases. MDR bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* have been associated with severe hospital-acquired infections and increased mortality rates [1].

In addition to antibiotic resistance, the ability of bacteria to form biofilms further complicates treatment strategies. Biofilms are structured communities of microorganisms embedded in a self-produced extracellular polymeric matrix that protects bacteria from antibiotics and host immune responses. Consequently, infections associated with biofilm formation are often chronic and difficult to eradicate [2].

Medicinal plants have long been recognized as valuable sources of bioactive compounds with antimicrobial properties. Among these, *Heliotropium indicum* has attracted attention due to its traditional medicinal uses and pharmacological potential. Extracts of the plant have been reported to exhibit antibacterial, antioxidant, anti-inflammatory, and wound-healing properties. Studies have demonstrated that the plant extract can inhibit several pathogenic bacteria and may also enhance the activity of existing antibiotics [3]. Because of its rich phytochemical composition and therapeutic potential, *Heliotropium indicum* represents a promising candidate for the development of new antibacterial and antibiofilm agents against MDR pathogens.

2. Material and Methodology

2.1 Botanical Description of *Heliotropium indicum*

Heliotropium indicum L. is an annual herb belonging to the family Boraginaceae. The plant is widely distributed in tropical and subtropical regions, including Asia, Africa, and South America. It commonly grows in open fields, roadsides, and wastelands. The plant typically reaches a height of 30–60 cm and possesses hairy stems and ovate leaves arranged alternately along the stem. The leaves are rough in texture and exhibit prominent veins. The plant produces small white or pale violet flowers arranged in curved inflorescences known as scorpioid cymes. The fruits are small nutlets containing seeds. Different parts of the plant such as leaves, stems, roots, and flowers have been used in traditional medicine for treating infections, wounds, skin diseases, and respiratory disorders [4].

3. Phytochemical Composition

The pharmacological activities of *Heliotropium indicum* are largely attributed to the presence of various bioactive secondary metabolites. Phytochemical investigations have revealed the presence of several classes of compounds including alkaloids, flavonoids, phenolic compounds, saponins, tannins, terpenoids, and carotenoids.

Among these, flavonoids and phenolic compounds are known for their strong antioxidant and antimicrobial properties. Alkaloids contribute to antimicrobial and anti-inflammatory activities, while tannins possess astringent and antibacterial effects. Terpenoids and saponins also play important roles in disrupting microbial cell membranes and inhibiting bacterial growth.

The synergistic interaction among these phytochemicals may enhance the overall antibacterial and antibiofilm activities of the plant [5].

4. Antibacterial Activity of *Heliotropium indicum*

4.1 Activity against Gram-Positive Bacteria

Several studies have demonstrated that extracts of *Heliotropium indicum* exhibit significant antibacterial activity against Gram-positive bacteria. Methanolic extracts of the plant have been shown to inhibit bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus* species.

These bacteria are responsible for various infections including skin infections, wound infections, and respiratory tract infections. The antibacterial activity may be attributed to phytochemicals that interfere with bacterial cell wall synthesis and metabolic processes [6].

4.2 Activity against Gram-Negative Bacteria

In addition to Gram-positive bacteria, the plant extract also exhibits inhibitory activity against Gram-negative pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella* species.

Gram-negative bacteria are often more resistant to antibiotics due to the presence of an outer membrane that acts as a protective barrier. However, bioactive compounds present in *Heliotropium indicum* may disrupt bacterial membranes, leading to leakage of cellular components and eventual cell death [7].

5. Antibiofilm Activity

Biofilm formation is recognized as one of the most significant mechanisms by which bacteria develop resistance to antibiotics and persist in hostile environments. A biofilm is a structured community of microbial cells that adhere to biotic or abiotic surfaces and are embedded within a self-produced extracellular polymeric substance (EPS) matrix composed of polysaccharides, proteins, lipids, and extracellular DNA. This matrix provides structural stability and protection to the bacterial community, allowing microorganisms to survive under adverse conditions such as exposure to antibiotics, disinfectants, and host immune responses. Bacteria living within biofilms exhibit significantly higher resistance to antimicrobial agents compared to their planktonic (free-floating) counterparts. This enhanced resistance is attributed to several factors, including restricted penetration of antibiotics through the biofilm matrix, altered metabolic activity of bacterial cells, and the presence of specialized persister cells that can survive antibiotic treatment. Consequently, biofilm-associated infections are often chronic,

recurrent, and difficult to eradicate, particularly in clinical settings involving medical devices such as catheters, implants, prosthetic joints, and wound dressings [8].

In recent years, medicinal plants have been extensively investigated as potential sources of antibiofilm agents capable of disrupting bacterial biofilm formation. Among these, *Heliotropium indicum* has attracted attention due to its diverse phytochemical composition and antimicrobial properties. Studies have suggested that extracts obtained from different parts of the plant possess the ability to inhibit biofilm formation in several pathogenic bacteria. The antibiofilm activity of *Heliotropium indicum* may occur through multiple mechanisms targeting different stages of the biofilm development process. The initial stage of biofilm formation involves the attachment of bacterial cells to a surface followed by colonization and formation of microcolonies. Bioactive compounds present in *Heliotropium indicum*, such as flavonoids, phenolic compounds, tannins, and alkaloids, may interfere with bacterial adhesion by altering cell surface properties or disrupting interactions between bacterial cells and host surfaces. By inhibiting this early adhesion phase, the plant extract can effectively prevent the establishment of biofilms [9].

In addition to preventing bacterial attachment, *Heliotropium indicum* extracts may also disrupt the extracellular polymeric matrix that stabilizes mature biofilms. The EPS matrix acts as a protective barrier that limits the penetration of antimicrobial agents and shields bacterial cells from environmental stress. Certain phytochemicals present in the plant may degrade or weaken components of the matrix, thereby destabilizing the structural integrity of the biofilm. As a result, bacterial cells become more exposed to antimicrobial agents and immune responses, leading to reduced bacterial survival and

biofilm dispersal. Furthermore, the plant extract may influence bacterial metabolic activity and reduce the production of extracellular substances required for biofilm maintenance.

Another important mechanism through which *Heliotropium indicum* may exert antibiofilm activity is by interfering with quorum sensing systems. Quorum sensing is a cell-to-cell communication mechanism used by bacteria to regulate gene expression in response to changes in population density. Through the production and detection of signaling molecules known as autoinducers, bacteria coordinate the expression of genes involved in biofilm formation, virulence factor production, and antibiotic resistance. Certain phytochemicals present in *Heliotropium indicum* may inhibit the synthesis or activity of these signaling molecules, thereby disrupting bacterial communication pathways. This inhibition prevents the coordinated expression of genes required for biofilm development and reduces bacteria's ability to form stable biofilm communities [10].

Overall, the antibiofilm potential of *Heliotropium indicum* highlights its importance as a promising natural source for combating biofilm-associated infections caused by multidrug-resistant bacteria. By targeting multiple stages of biofilm formation, including bacterial adhesion, extracellular matrix stability, and quorum-sensing communication, the bioactive compounds present in the plant may significantly reduce bacterial colonization and persistence. These properties suggest that *Heliotropium indicum* extracts could be further explored for developing novel antibiofilm agents, particularly for the prevention and treatment of chronic infections and medical device-associated bacterial colonization.

6. Mechanisms of Antibacterial and Antibiofilm Action

The antibacterial and antibiofilm activities of *Heliotropium indicum* are believed to involve several mechanisms:

6.1 Disruption of Bacterial Cell Membranes

One of the primary mechanisms through which *Heliotropium indicum* exhibits antibacterial activity is the disruption of bacterial cell membranes. The bacterial cell membrane plays a crucial role in maintaining cellular integrity, regulating nutrient transport, and protecting the cell from environmental stress. Bioactive compounds present in *Heliotropium indicum*, particularly terpenoids, phenolic compounds, and certain alkaloids, have the ability to interact with the lipid components of bacterial membranes. These phytochemicals may alter membrane permeability by destabilizing the phospholipid bilayer structure. As a result, essential intracellular components such as ions, proteins, nucleotides, and metabolites begin to leak out of the cell. This leakage disrupts vital cellular processes, ultimately leading to loss of membrane integrity and bacterial cell death. Furthermore, membrane damage may impair the function of membrane-bound proteins and enzymes involved in respiration and energy production. The ability of plant-derived compounds to target bacterial membranes is particularly advantageous because it reduces the likelihood of resistance development. Therefore, the membrane-disrupting properties of *Heliotropium indicum* phytochemicals contribute significantly to its antibacterial and antibiofilm activities [11].

6.2 Inhibition of Enzymatic Activity

Another important mechanism responsible for the antibacterial effects of *Heliotropium indicum* involves the inhibition of bacterial

enzymatic activity. Bacteria rely on numerous enzymes to carry out essential metabolic processes such as DNA replication, protein synthesis, energy production, and cell wall biosynthesis. Phytochemicals present in the plant extract may interfere with the function of these enzymes by binding to their active sites or altering their structural configuration. For instance, flavonoids and phenolic compounds have been reported to inhibit enzymes involved in nucleic acid synthesis and bacterial metabolism. Such inhibition prevents the normal functioning of metabolic pathways necessary for bacterial survival and proliferation. In addition, certain compounds may inhibit enzymes responsible for cell wall synthesis, thereby weakening the bacterial structure and making the cells more susceptible to damage. By targeting multiple enzymatic pathways simultaneously, plant-derived bioactive compounds can effectively suppress bacterial growth and reduce the viability of pathogenic microorganisms [12].

6.3 Interference with Quorum Sensing

Quorum sensing is a sophisticated communication mechanism used by bacteria to coordinate collective behavior in response to changes in population density. Through this process, bacteria release and detect signaling molecules known as autoinducers, which regulate gene expression related to virulence, biofilm formation, toxin production, and antibiotic resistance. The ability to form biofilms through quorum sensing allows bacterial communities to survive under adverse conditions and resist antimicrobial treatments. Compounds present in *Heliotropium indicum* may interfere with quorum sensing pathways by inhibiting the production or activity of these signaling molecules. Certain phytochemicals can block receptor sites responsible for detecting autoinducers, thereby preventing the

transmission of communication signals among bacterial cells. As a result, the coordinated expression of genes involved in biofilm formation and virulence is disrupted. This interference significantly reduces bacterial adhesion, colonization, and the formation of protective biofilm structures. Consequently, pathogens become more vulnerable to antimicrobial agents and host immune responses. The quorum sensing inhibitory activity of *Heliotropium indicum* represents an important strategy for controlling infections caused by multidrug-resistant bacteria [13].

6.4 Synergistic Interaction with Antibiotics

In addition to its direct antibacterial effects, *Heliotropium indicum* may also enhance the activity of conventional antibiotics through synergistic interactions. Multidrug-resistant bacteria often possess mechanisms such as efflux pumps, enzyme production, and reduced membrane permeability that decrease the effectiveness of antibiotics. Certain phytochemicals present in the plant extract may overcome these resistance mechanisms and restore antibiotic sensitivity. For example, some plant compounds can increase bacterial membrane permeability, allowing antibiotics to penetrate more easily into the bacterial cell. Others may inhibit efflux pumps, which are specialized proteins that expel antimicrobial agents from the bacterial cell. By blocking these pumps, the intracellular concentration of antibiotics increases, leading to improved antibacterial activity. Additionally, phytochemicals may weaken bacterial defense mechanisms, making pathogens more susceptible to antibiotic treatment. The synergistic interaction between plant-derived compounds and conventional antibiotics offers a promising approach for combating infections caused by multidrug-resistant bacteria and may contribute to the development of combination therapies in the future [14].

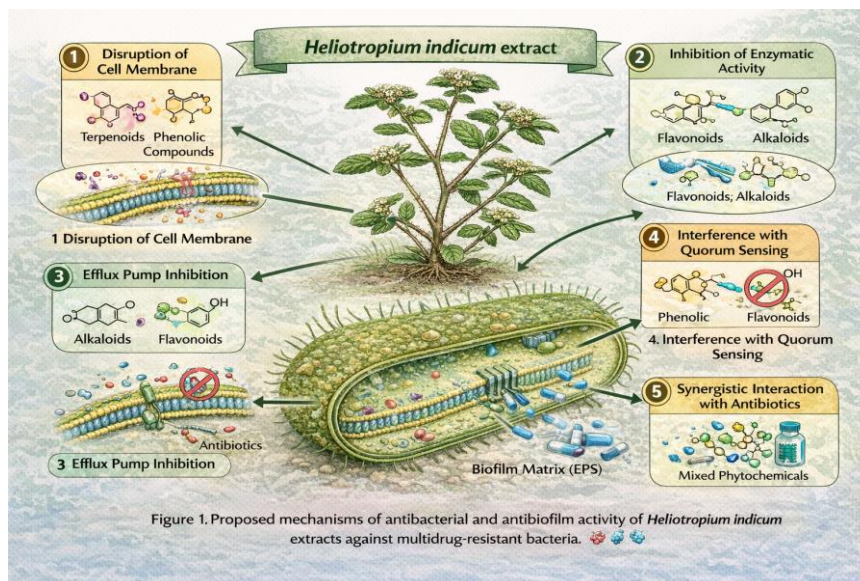


Table 1. Mechanisms of antibacterial and antibiofilm action of bioactive compounds from *Heliotropium indicum*

Mechanism of Action	Bioactive Compounds Involved	Target in Bacterial Cell	Effect on Bacteria
Disruption of bacterial cell membrane	Terpenoids, phenolic compounds	Phospholipid bilayer of bacterial membrane	Increases membrane permeability leading to leakage of intracellular contents and cell death
Inhibition of enzymatic activity	Flavonoids, alkaloids	Metabolic enzymes involved in DNA replication and protein synthesis	Blocks essential metabolic pathways and inhibits bacterial growth
Interference with quorum sensing	Phenolic compounds, flavonoids	Quorum sensing signaling molecules and receptors	Prevents bacterial communication and biofilm formation
Inhibition of biofilm matrix formation	Tannins, saponins	Extracellular polymeric substances (EPS)	Disrupts structural stability of biofilms
Efflux pump inhibition	Alkaloids, flavonoids	Bacterial efflux pump proteins	Prevents expulsion of antibiotics from bacterial cells
Synergistic interaction with antibiotics	Mixed phytochemicals	Bacterial membrane and resistance mechanisms	Enhances antibiotic penetration and restores antibiotic sensitivity

7. Potential Applications

The antibacterial and antibiofilm properties of *Heliotropium indicum* highlight its significant potential in various medical and pharmaceutical applications, particularly in addressing the growing problem of multidrug-resistant (MDR) bacterial infections. As antibiotic resistance continues to threaten global healthcare systems, the search for alternative therapeutic agents from natural sources has intensified. Medicinal plants have historically served as valuable sources of bioactive compounds used in modern drug development, and *Heliotropium indicum* represents a promising candidate due to its rich phytochemical composition. Extracts obtained from different parts of the plant, including leaves, stems, and roots, contain secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds that exhibit potent antimicrobial activities. These compounds may inhibit bacterial growth through multiple mechanisms, including disruption of bacterial membranes, inhibition of metabolic enzymes, and interference with quorum sensing pathways involved in biofilm formation [15].

One of the most important potential applications of *Heliotropium indicum* lies in the development of natural antimicrobial agents for the treatment of infections caused by MDR bacteria. Pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* have developed resistance to many commonly used antibiotics, making infections increasingly difficult to treat. Plant-derived antimicrobial compounds may provide alternative therapeutic strategies with fewer side effects and reduced risk of resistance development. Extracts of *Heliotropium indicum* may therefore be incorporated into herbal formulations, topical

treatments, or oral medications aimed at controlling bacterial infections [16].

In addition to their direct antibacterial effects, the bioactive compounds isolated from *Heliotropium indicum* may also serve as lead molecules for the development of novel antibiotics. Modern pharmaceutical research often relies on natural products as templates for the synthesis of new drugs. By isolating and characterizing the active phytochemicals responsible for antibacterial activity, researchers may be able to design more potent antimicrobial agents capable of targeting resistant bacterial strains. Furthermore, some plant compounds may function as antibiotic adjuvants, enhancing the effectiveness of conventional antimicrobial drugs. For example, certain phytochemicals may increase bacterial membrane permeability or inhibit efflux pumps that normally expel antibiotics from bacterial cells. This synergistic interaction can restore the activity of existing antibiotics against resistant bacteria [17].

Another promising application of *Heliotropium indicum* extracts is in the prevention and control of biofilm-associated infections. Biofilms are structured microbial communities that adhere to surfaces and produce a protective extracellular matrix, making them highly resistant to antibiotics and immune responses. Biofilm formation is commonly associated with infections related to medical devices such as catheters, implants, prosthetic joints, and contact lenses. The antibiofilm properties of *Heliotropium indicum* may be utilized in the development of antimicrobial coatings for medical devices. Incorporating plant-derived compounds into device surfaces could help prevent bacterial adhesion and biofilm formation, thereby reducing the risk of device-associated infections [18].

Furthermore, the plant extract may be incorporated into wound dressings and topical formulations designed to treat infected wounds and prevent microbial colonization. Chronic wounds often involve biofilm-forming bacteria that delay the healing process and increase the risk of complications. Natural antimicrobial compounds derived from *Heliotropium indicum* may help control bacterial growth, reduce inflammation, and promote wound healing. The integration of such plant-based antimicrobial agents into modern biomedical materials could represent a promising strategy for improving infection control in clinical settings [19].

Overall, the diverse antibacterial and antibiofilm activities of *Heliotropium indicum* suggest that this medicinal plant holds considerable promise for future therapeutic and pharmaceutical applications. Continued research focusing on the isolation, characterization, and clinical evaluation of its bioactive compounds will be essential to fully explore its potential in combating multidrug-resistant bacterial infections and biofilm-associated diseases [20].

Table 2. Antibacterial Activity of *Heliotropium indicum* Extracts against Selected Multidrug-Resistant Bacteria

Bacterial Species	Type of Bacteria	Common Infection	Observed Activity of Plant Extract
<i>Staphylococcus aureus</i>	Gram-positive	Skin infections, wound infections	Strong antibacterial activity
<i>Escherichia coli</i>	Gram-negative	Urinary tract infections	Moderate antibacterial activity
<i>Pseudomonas aeruginosa</i>	Gram-negative	Hospital-acquired infections	Moderate to strong inhibition
<i>Klebsiella pneumoniae</i>	Gram-negative	Pneumonia, bloodstream infections	Moderate antibacterial effect
<i>Bacillus subtilis</i>	Gram-positive	Food contamination	Strong antibacterial activity

8. Conclusion

The rapid emergence and global spread of multidrug-resistant (MDR) bacterial pathogens have become a major challenge for modern healthcare systems. The overuse and misuse of conventional antibiotics have accelerated the development of resistance mechanisms among pathogenic bacteria, leading to reduced effectiveness of existing antimicrobial drugs. In addition to antibiotic resistance, the ability of bacteria to form biofilms further complicates treatment strategies because biofilm-associated bacteria exhibit enhanced tolerance to antimicrobial agents and host immune responses. Consequently, infections caused by MDR and biofilm-forming bacteria are often persistent, difficult to treat, and associated with increased morbidity and mortality. These challenges have intensified the search for alternative antimicrobial agents derived from natural sources, particularly medicinal plants that possess diverse bioactive compounds with therapeutic potential.

Among the various medicinal plants studied for antimicrobial properties, *Heliotropium indicum* has attracted considerable scientific attention due to its rich phytochemical composition and traditional use in the treatment of infections and inflammatory conditions. Phytochemical analyses have revealed that different parts of the plant contain a wide variety of secondary metabolites, including flavonoids, alkaloids, tannins, phenolic compounds, saponins, and terpenoids. These bioactive compounds are known to exhibit a broad spectrum of biological activities, including antibacterial, antioxidant, anti-inflammatory, and wound-healing effects. Several experimental studies have demonstrated that extracts of *Heliotropium indicum* possess significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including clinically important pathogens

such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* species. The antibacterial effects of the plant are believed to occur through multiple mechanisms, such as disruption of bacterial cell membranes, inhibition of essential metabolic enzymes, interference with quorum sensing systems, and suppression of biofilm formation.

In addition to its direct antibacterial effects, *Heliotropium indicum* has also shown promising antibiofilm activity. Biofilm formation plays a crucial role in bacterial survival and persistence, particularly in chronic infections and medical device-associated infections. The phytochemicals present in the plant may inhibit the initial adhesion of bacterial cells to surfaces, disrupt the extracellular polymeric matrix that stabilizes biofilms, and interfere with quorum sensing pathways responsible for bacterial communication and coordinated biofilm development. By targeting these multiple stages of biofilm formation, plant-derived compounds may significantly reduce bacterial colonization and enhance the effectiveness of antimicrobial treatments.

Despite the promising results obtained from in vitro and preliminary experimental studies, further research is necessary to fully explore the therapeutic potential of *Heliotropium indicum*. Future investigations should focus on the isolation, purification, and structural characterization of the specific bioactive compounds responsible for its antibacterial and antibiofilm activities. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and nuclear magnetic resonance (NMR) spectroscopy may be employed to identify these compounds. In addition, comprehensive studies on toxicity, pharmacokinetics, pharmacodynamics, and

safety profiles are essential to ensure the safe use of plant-derived compounds in clinical applications. Well-designed in vivo experiments and clinical trials are also required to evaluate the efficacy of these compounds in treating infections caused by MDR bacteria.

Overall, *Heliotropium indicum* represents a valuable medicinal plant with considerable potential for the development of novel antibacterial and antibiofilm agents. Its diverse phytochemical constituents and multiple mechanisms of action make it a promising candidate for future pharmaceutical research aimed at combating multidrug-resistant bacterial infections. Continued scientific investigation and interdisciplinary research efforts may ultimately lead to the development of new plant-based antimicrobial therapies that can help address the growing global problem of antibiotic resistance.

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