ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC ACTINOBACTERIA ASSOCIATED WITH SELECTED TRADITIONAL MEDICINAL PLANTS AGAINST CLINICAL MDR PATHOGENS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

LALROKIMI

MZU REGISTRATION NO.: 1703966

Ph. D. REGISTRATION NO.: MZU/Ph. D./1425 of 26.07.2019

DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF LIFE SCIENCES

JULY 2025

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BY

LALROKIMI

Department of Biotechnology

Supervisor: Dr. ESTHER LALNUNMAWII

Joint Supervisor: Dr. ZOTHANPUIA

Submitted

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Biotechnology of Mizoram University, Aizawl

DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF LIFE SCIENCES
MIZORAM UNIVERSITY
(A Central University)
TANHRIL, AIZAWL – 796004: MIZORAM, INDIA

Dr. Esther Lalnunmawii
Assistant Professor **E-mail: essiezd.mzu@gmail.com**

CERTIFICATE

This is to certify that the thesis entitled **"Antimicrobial potential of endophytic actinobacteria associated with selected traditional medicinal plants against clinical MDR pathogens"** submitted by **Lalrokimi**, Ph.D. Research Scholar for the award of the Degree of Doctor of Philosophy in Biotechnology is carried out under my supervision and incorporates the student bonafide research and this has not been submitted for the award of any degree in this or any other university or institute of learning.

Place: Aizawl, Mizoram
Date:

(Dr. ESTHER LALNUNMAWII)
Supervisor

(Dr. ZOTHANPUIA)
Joint Supervisor

DECLARATION
MIZORAM UNIVERSITY
JULY, 2025

I Lalrokimi, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Biotechnology.

(LALROKIMI)
Research Scholar

(PROF. N SENTHIL KUMAR)
Head of the Department

(Dr. ESTHER LALNUNMAWII)
Supervisor

ACKNOWLEDGEMENT

I would like to begin by expressing my heartfelt gratitude to the Almighty God for blessing me with good health and the unwavering strength that has enabled me to undertake and successfully complete this research endeavour.

I extend my deepest appreciation to my supervisor, Dr. Esther Lalnunmawii, and my co-guide, Dr. Zothanpuia. Their invaluable guidance, steadfast support, and generous provision of essential resources have been instrumental in the successful completion of my research work. Their expertise and encouragement have inspired me throughout this journey, and for that, I am truly thankful.

I would like to express my heartfelt gratitude to the Department of Biotechnology in New Delhi for the pivotal establishment of the DBT-BIF centre and the DBT-State Biotech Hub within our Department. This initiative has laid a strong foundation for our research endeavours. I am equally appreciative of the Department of Science and Technology in New Delhi for their generous financial support, which has been vital in advancing our projects.

I extend my sincere thanks to Prof. N. Senthil Kumar, the esteemed Head of the Department of Biotechnology at Mizoram University, for his unwavering cooperation and guidance. Additionally, I am grateful to all the faculty members and technical staff of the Department, whose constant support and expertise have greatly enriched my research journey.

I am especially thankful to my dedicated lab members Purbajyoti Deka, Yogesh Malvi, Lallawmsangi, William Carrie, and Suman Nayak whose friendship and collaboration have made this challenging work not only possible but also enjoyable.

A special acknowledgment goes to my husband, Malsawmzuala, whose priceless assistance in sample collection and in drafting my papers has been instrumental to my progress and for providing me with an endless emotional support.

Lastly, I wish to convey my deepest appreciation to my mother, R. Zahmingthangi, whose unwavering support has been my greatest strength and inspiration. I also want to thank my siblings Lalropuii, Lalrosangpuii and Lalrohlupuii, whose encouragement has been a source of motivation, enveloping me in their boundless love, prayers, and moral support. Their belief in me has played a crucial role in my research accomplishments.

Place: Aizawl **(LALROKIMI)**

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LIST OF ABBREVIATIONS

MDR	Multi Drug Resistant
WHO	World Health Organisation
MRSA	Methicillin Resistant *Staphylococcus aureus*
VRE	Vancomycin Resistant *Enterococcus*
CDC	Centers for Disease Control and Prevention
MDRTB	Multi Drug Resistant Tuberculosis
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
GCMS	Gas Chromatography-Mass Spectrometry
UV	Ultraviolet
TB	Tuberculosis
HA-MRSA	Hospital-acquired Methicillin-Resistant *Staphylococcus aureus*
HIV	Human immunodeficiency viruses
SCNA	Starch Casein Nitrate Agar
TWYE	Tap Water Yeast Extract
YECA	Yeast Extract Casamino Acid

DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic acid
ROS	Reactive Oxygen Species
HAT	Hydrogen Atom Transfer
SET	Single Electron Transfer
HPTLC	High Performance Thin Layer Chromatography
TLC	Thin Layer Chromatography
SCA	Starch Casein Agar
AIA	Actinomyces Isolation Agar
ISP5	Glycerol Asparagine Agar Base
ISP7	Tyrosine Agar
ISP1	Tryptone Yeast Extract Broth
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
MIC	Minimum Inhibitory Concentration
VOC	Volatile Organic Compound
CFU	Colony Forming Units
SEM	Scanning Electron Microscope

CHAPTER 1

INTRODUCTION

And

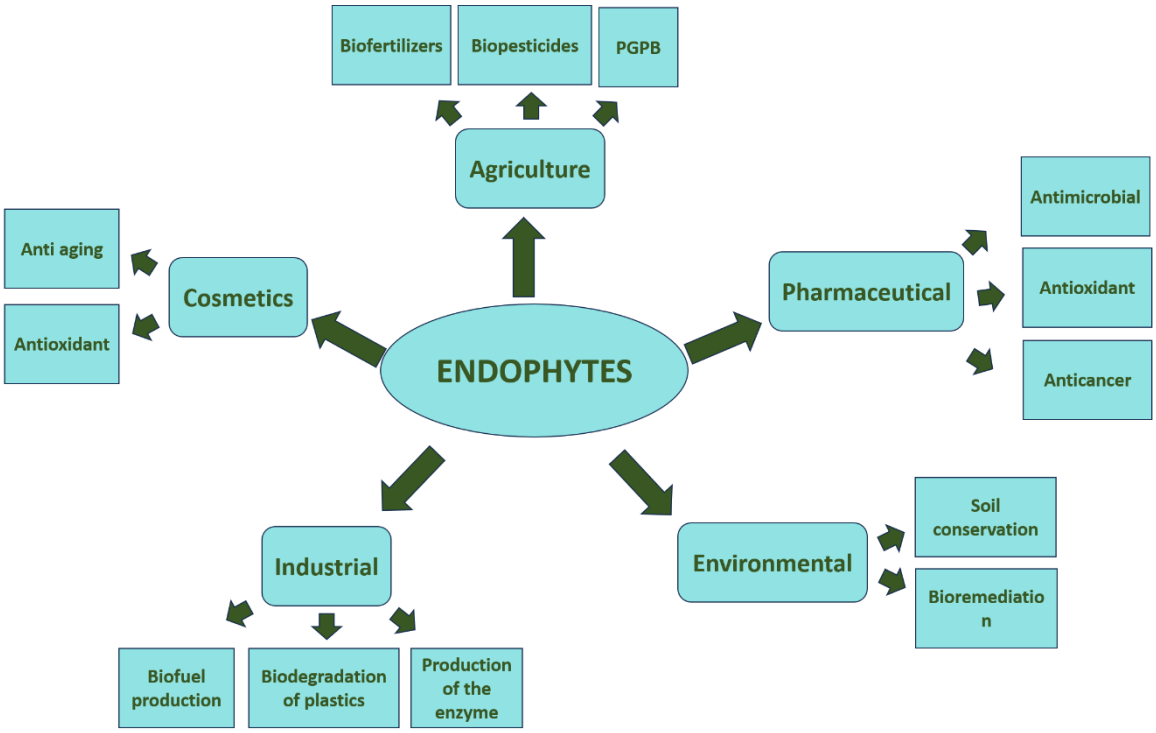
REVIEW OF LITERATURE

INTRODUCTION

Endophytes are beneficial microorganisms inhabiting the tissues of plants and have a positive effect on the plants without causing any harm. They play an important role in promoting plant growth as well as the ability to tolerate abiotic stress and enhancing overall vitality while maintaining a symbiotic relationship with their hosts (Wippel, 2023). Numerous microbial populations, including fungi and bacteria, have been recognized as endophytes residing within the internal tissues of plants. These symbiotic organisms play significant roles in plant health and stress resilience, contributing to a deeper understanding of plant-microbe interactions ecologically and agriculturally (Schulz and Boyle 2006). The term endophyte was coined by De Bary (1866). Nearly all plants on our planet have been discovered to harbour one or more endophytes, as highlighted in the research by Petrini et al. (1992). These fascinating microorganisms are remarkably widespread and exhibit a rich biodiversity, having been identified in every plant species studied so far. Remarkably, of the approximately 300000 plant species that populate the Earth, each individual plant serves as a host to a variety of endophytes, highlighting the intricate relationships that exist within our ecosystems (Strobel and Daisy, 2003). The microorganisms that inhabit plant tissues, predominantly in a symbiotic relationship, encompass various groups, such as fungi and bacteria, including actinobacteria (Pimentel et al., 2011; Singh and Dubey, 2015). Endophytes complete their life cycles within host plants, without causing any harm to them. These organisms are widely distributed in nature and establish specific associations with their host plants, engaging in mutualism or antagonism, but not parasitism (Nair and Padmavathy, 2014). The role endophytes play is crucial for the growth of the host plant by producing phytohormones and other growth-enhancing factors. In return, they receive nutrients and habitat from the host plant. Furthermore, endophytes contribute to the host's adaptability against abiotic and biotic stresses while providing protection against pests, insects and pathogens.

Considering the unique colonization patterns observed in specific host plants, researchers estimate that there could be as many as 1 million distinct endophyte species. However, only a small fraction of these have been documented and characterized (Petrini et al. 1992). This leaves vast potential for discovering new and

valuable natural products derived from intriguing endophytic microorganisms that inhabit a diverse range of plants across various niches and ecosystems. The unexplored diversity of these microorganisms presents a significant opportunity for future research and innovation in bioprospecting. Many endophytes have the ability to produce bioactive compounds that help plants defend against pathogens. Some of these compounds have also shown promise in novel drug discovery. Recent studies have identified various natural products derived from endophytes, including flavonoids, alkaloids, terpenoids and steroids. To date, the majority of natural products sourced from endophytes are classified as antibiotics, anticancer agents, biological control agents, and other bioactive compounds, each serving various functional roles.



Actinobacteria are generally filamentous bacteria that are Gram-positive, and they represent a significant phylum within the domain of Bacteria (Ludwig and Klenk 2005). Actinobacteria are a diverse group of microorganisms that can be found thriving in various ecosystems, both on land and in aquatic environments. These fascinating organisms predominantly exist as free-living entities, showcasing their remarkable adaptability to a wide range of habitats. Among these environments, soil stands out as the primary reservoir for actinobacteria, serving as a rich ground for their interactions,

particularly with the root systems of plants. Research indicates that these endophytic actinobacteria are particularly abundant in the roots, where they engage in vital symbiotic relationships with plants. They are present in moderate numbers within the stems, while their occurrence in the leaves is notably less frequent. This distribution highlights the critical role that actinobacteria play in plant health and soil ecosystems, emphasizing their importance within the complex ecosystem that exists beneath the surface. (Gangwar et al., 2014). This distribution in growth pattern for endophytic actinobacteria appears reasonable, as the roots are most exposed to and interact extensively with the microbial community in the rhizosphere. These organisms are crucial in breaking down complex materials derived from deceased plants, algae, fungi and animals. Through their activities, they facilitate the decomposition process, transforming these complex organic substances into simpler compounds. This natural recycling not only returns essential nutrients back to the ecosystem but also contributes to the formation of humus, enriching the soil and promoting healthy plant growth. (Sharma 2014). Actinobacteria represent a significant and diverse group of microorganisms renowned for their remarkable capability to synthesize a variety of bioactive metabolites. These metabolites include a wide range of compounds that possess powerful antimicrobial properties, potential anti-cancer effects, and various other pharmaceutical applications. Their unique biochemical pathways make them a valuable resource in the development of new drugs and therapeutic agents, highlighting their importance in microbiology and pharmaceutical research (Schulz et al. 2009; Barka et al., 2016). These microorganisms have been the main source of various antibiotics since the discovery of Penicillin 1928 contributing a rich diversity of antibiotics to combat many lethal diseases. The total count of bioactive metabolites generated by microorganisms is approximately 23,000, with actinobacteria alone producing 10,000 of these (which represents 45% of all bioactive metabolites), and from this group, a remarkable 7,600 (accounting for 76%) of compounds have been identified from a single genus, Streptomyces (Berdy 2012). This highlights their critical significance in the pharmaceutical industry.

For many centuries, plants have served as vital sources of bioactive compounds utilized for their therapeutic properties. These natural resources have been revered for

their ability to help in healing and health maintenance. In recent years, researchers have uncovered the remarkable role of microorganisms associated with these plants. These microorganisms have demonstrated the capacity to produce a diverse range of compounds, many of which possess significant therapeutic value, opening new pathways for medicinal applications and enhancing our understanding of plant-microbe interactions in health and wellness (Subbulakshmi et al., 2012).

For decades, antibiotics have played a crucial role in treating and controlling microbial infections, saving countless lives. However, as their use has become more widespread over time, there has also been a significant rise in the overuse and misuse of these powerful medications. This escalating misuse has led to the alarming emergence and proliferation of antibiotic-resistant bacteria. These resilient strains pose a serious threat to public health, accounting for thousands of fatalities each year as they render standard treatments ineffective and complicate the management of infections (Jørgensen et al., 2017). Numerous studies have highlighted the emerging ability of various microorganisms such as bacteria, parasites, viruses, and fungi to develop resistance to treatments. This alarming trend indicates that antibiotics, which were once effective in combating these pathogens, are increasingly losing their potency and efficiency in medical use (Alexander et al., 2013; Mediavilla et al., 2016). Multidrug resistance (MDR) occurs when a single strain of bacteria develops the ability to withstand the effects of multiple antibiotics. This resistance undermines the effectiveness of these antibiotics, which once successfully inhibited or eradicated the pathogens. As a result, infections caused by these resistant bacteria can persist within the body, complicating treatment efforts and significantly increasing the risk of transmission to others. The implications of MDR infections extend beyond individual health, often leading to higher rates of morbidity and mortality, as well as longer hospital stays and more complex medical interventions (de Kraker et al., 2011). Moreover, MDR has emerged as a pressing global health concern, representing a profound threat to both human and animal health. Its widespread impact poses significant challenges to public health systems and the economy, highlighting the urgent need for effective strategies to combat these resistant infections (O'Neill, 2017).

Bacteria are capable of undergoing mutations that grant them resistance to antibiotics, making the infections they cause increasingly challenging to treat. According to the World Health Organization (WHO), a staggering 700,000 individuals succumb to drug-resistant diseases every year. Of these, 230,000 people are victims of multidrug-resistant tuberculosis, a serious and often life-threatening infection. Many conditions related to the urinary and respiratory tracts, as well as sexually transmitted infections, are evolving into forms that challenge conventional treatments. Among communicable diseases, lower respiratory infections have emerged as the most lethal, claiming the lives of approximately 3.0 million people globally, with a disproportionate impact on populations in low-income countries. The growing prevalence of these resistant infections has led to a significant public health threat, underscoring the urgent need for innovative solutions and strategies to combat antibiotic resistance.

The rise of multidrug resistance can primarily be due to the excessive and inappropriate use of antimicrobial agents for controlling, treating, and preventing diseases. The widespread use of these powerful medications in livestock, along with the misuse of prescribed antibiotics in humans, has significantly increased the growing problem of antimicrobial resistance. This surge in resistance not only complicates treatment options but also poses a serious threat to public health and the efficacy of existing medications (Brinkac et al., 2017). With the rise in the usage of antimicrobials, the landscape of bacterial pathogens has become increasingly complicated, as these organisms develop competent mechanisms to resist treatment. Scientists face significant challenges in their efforts to combat infections, fighting against the ever-evolving nature of these microbes. The pace at which new antimicrobial agents are being developed is unable to keep up with the rapid emergence of resistance, as microorganisms continuously adapt and refine their survival strategies. This ongoing battle highlights the pressing need for innovative approaches to address bacterial resistance and protect public health (Krause, 1992). Alarmingly high resistance rates for certain healthcare-associated infections are on the rise, with methicillin-resistant *Staphylococcus aureus* (MRSA) reaching an astounding 97%, vancomycin-resistant *Enterococcus* (VRE) at 59%, and multidrug-resistant tuberculosis (MDR-TB) standing at 52% as reported by the CDC in 2019. The widespread and often indiscriminate use

of methicillin, an antibiotic once considered effective against staphylococcal infections, has paved the way for the emergence of resistant strains. These resistant bacteria are now responsible for numerous skin infections globally, leading to serious health concerns. The root of this resistance can be traced back to staphylococcal cassette chromosome mec (SSCmec) which is a mobile genetic element. This element harbours the methicillin resistance gene (mecA), which bacteria acquire through a process called conjugation that is a form of genetic exchange among closely related bacterial species. The mecA gene encodes for an enzyme that modifies the target site for beta-lactam antibiotics, specifically by altering the peptidoglycan-binding proteins in the bacterial cell wall. This binding enzyme, known as the methicillin-resistant penicillin-binding protein, plays a crucial role in the cross-linking of peptidoglycan, a vital component of the bacterial cell wall structure. The modification significantly diminishes the effectiveness of beta-lactam antibiotics, rendering them nearly useless against these resistant strains (Harkins et al., 2017). Consequently, the rapid proliferation of multidrug-resistant organisms has escalated the urgency to discover and develop new antibiotics that can effectively combat these formidable pathogens and safeguard public health.

Numerous researchers have documented the diversity of endophytes, with a particular emphasis on endophytic actinobacteria. Among the various species identified, Streptomyces becomes the most predominant group. These microorganisms play an important role in their host plants, contributing to various ecological and biochemical processes. The prevalence of Streptomyces in endophytic communities highlights their significance in promoting plant health and enhancing resilience against environmental stressors (Matsukuma et al. 1994; Matsumoto et al. 1998; Kurtboke 2003). Research on the antioxidant activity of Streptomyces has shown promising results. A comprehensive study conducted by Rammali et al. in 2024 investigated into antimicrobial and antioxidant properties of a specific strain of actinobacteria, *Streptomyces* sp. EIZ2, which was isolated from agricultural soil in Morocco. The research findings highlighted that the extract derived from *Streptomyces* sp. EIZ2 exhibited a significant antioxidant capability, effectively neutralizing DPPH and ABTS free radicals. Furthermore, the extract demonstrated a significant enhancement in

ferric-reducing antioxidant power, exhibiting its potential as a strong antioxidant agent. The identification of a positive correlation between antioxidant activities and the concentrations of total phenolic and flavonoid compounds present in the extract are important since these compounds are well known for their health benefits, particularly in combating oxidative stress.

Extensive research has revealed that various species within the genus Streptomyces possess the remarkable ability to synthesize a wide range of bioactive compounds, that exhibit potent antimicrobial properties. These compounds are effective against a diverse range of pathogens, including gram negative and positive bacteria, fungi, and even viruses. For example, *Streptomyces coelicolor*, a well-studied species within this genus, is known for producing a striking blue pigment called actinorhodin. This substance has demonstrated significant antimicrobial activity, showcasing effectiveness against multiple strains of bacteria. Similarly, another notable species, *Streptomyces griseus*, has gained recognition for its production of streptomycin, a powerful antibiotic that plays a crucial role in the treatment of various bacterial infections. These discoveries underscore the immense potential of *Streptomyces* species in drug development and the broader field of antimicrobial research (Berdy 2005). In a separate investigation, researchers explored the antimicrobial properties of a methanolic extract derived from *Streptomyces* sp. This study evaluated the extract's effectiveness against various microorganisms, including various strains of bacteria, fungi, and yeast. The findings revealed that the methanolic extract exhibited significant antimicrobial activity across all tested organisms, with particularly strong effects observed against Gram-positive bacteria. These promising results suggest that the methanolic extract from *Streptomyces* sp. holds great potential as a natural antimicrobial agent, suitable for further development in applications aimed at combating microbial infections (Devi et al., 2023).

In the study performed by Kumar et al. in 2013, a comprehensive analysis was performed on the methanolic extract derived from the microbial genus Streptomyces. Utilizing Gas Chromatography-Mass Spectrometry (GC-MS) as a key analytical technique, the researchers revealed a diverse range of bioactive compounds present in the extract. Among these, several significant components were identified, including 2-phenylethanol, benzeneethanol, acetic acid, butanedioic acid, hexadecanoic acid, and

9-octadecenoic acid. Each of these compounds is recognized for its distinct biological activities. For instance, both 2-phenylethanol and benzeneethanol have shown significant antimicrobial and antifungal properties, indicating their potential utility in combating various microbial infections. Meanwhile, hexadecanoic acid and 9-octadecenoic acid have garnered attention for their dual roles as antimicrobial agents and antioxidants, which can contribute to cellular protection against oxidative stress. The findings of this study suggest that the methanolic extract from *Streptomyces* sp. could possess considerable therapeutic potential, meriting further investigation to explore its applications in health and medicine. The diverse pharmacological activities highlighted by the identified compounds pave the way for potential innovations in natural product drug development.

Terpenoids represent a diverse group of natural compounds derived from isoprene, characterized by their complex structures and wide-ranging functions. These remarkable molecules are vital for plants, as they help them withstand various stresses, both biotic like pest attacks and diseases and abiotic, such as extreme temperatures and drought. Additionally, terpenoids play an essential role in facilitating interactions between plants and other organisms, particularly in attracting pollinators with their appealing scents. Beyond their ecological significance, terpenoids also possess beneficial properties for human health, including the ability to lower blood pressure, and they are utilized as natural insecticides, showcasing their versatility and importance in both nature and agriculture (Kabera et al., 2014). Alkaloids represent a class of nitrogen-containing cyclic compounds that play an important role in various biological processes. These fascinating molecules serve as foundational scaffolds for several important antibiotics, including metronidazole and quinolones. Their unique chemical structures contribute to their effectiveness in treating bacterial infections and highlight the significant role alkaloids play in pharmacology (Cushnie et al., 2014). Flavonoids are complex compounds characterized by a polyphenolic structure, which is synthesized via the polypropanoid pathway (Ghasemzadeh and Ghasemzadeh, 2011). These unique compounds have gained recognition for their remarkable biological properties, serving as potent antioxidants that combat oxidative stress in the body. Their anticancer potential has gathered considerable attention, as they exhibit the ability to inhibit the growth of cancer cells. Furthermore, flavonoids have demonstrated

antibacterial activity, making them valuable in fighting various infections. Additionally, they play a vital role in promoting heart health by offering cardioprotective benefits. Beyond these internal health benefits, flavonoids also contribute to skin protection by shielding it from harmful UV radiation, highlighting their complex roles in health and wellness (Tungmunnithum et al., 2018).

Plants are remarkable organisms that not only supply essential minerals and primary metabolites but also produce a diverse array of secondary metabolites. These include complex compounds such as flavonoids, alkaloids, saponins, terpenoids, phenolics, and lipids, all of which are known to possess significant therapeutic potential (Sivasankaridevi et al., 2013). The phytochemicals synthesized by plants play a vital role in their interactions with the environment, especially with the endophytic organisms. These endophytes, which can be fungi or bacteria living within the plant tissues, often form intricate relationships with their host plants, influencing not just the plant's health and resilience but also the overall quality and effectiveness of these valuable secondary metabolites. (Egamberdieva et al., 2017). Extensive research has revealed the significant role that endophytes play in enhancing the medicinal properties of ethnobotanical plants. In some cases, the secondary metabolites produced by these endophytic organisms closely reflect those found in their host plants, positioning them as promising candidates for drug development. A notable example is found within the Taxaceae family, where the endophyte *Pestalotiopsis microspora* has gained recognition for its ability to produce torreyanic acid, a metabolite that closely resembles that of its plant host. This particular compound is well known for its potent anticancer properties, highlighting the potential of endophytes in therapeutic applications (Ludwig-Müller, 2015). Unlike primary metabolites, which are directly associated to the growth and development of an organism, secondary metabolites are organic compounds that serve crucial functions in plant defence mechanisms and enable plants to adapt effectively to their environmental conditions. These intricate relationships illustrate the profound interconnectedness of endophytes and their host plants in the realm of natural medicine (Tiwari and Rana, 2015).

Since microbial life is ubiquitous in the tissues of medicinal plants, these organisms serve as an invaluable reservoir of therapeutic agents. Their prevalence, along with their remarkable ability to produce bioactive metabolites that reflect those found in the

plants they inhabit, underscores their potential for pharmaceutical applications. Endophytic actinobacteria, in particular, can be cultured in nutrient media, thriving even in extreme conditions where the host plants might struggle to survive. This resilience positions them as promising candidates for the discovery of novel drugs. Despite this potential, a significant gap in research regarding the novelty, diversity, and bioactivity of endophytic actinobacteria sourced from traditional medicinal plants, especially in biodiversity-rich regions still remain. To address this gap, the current study aims to explore the traditional medicinal plants native to Mizoram, located in Northeast India—a region recognized as part of the Indo-Burma biodiversity hotspot. The objective is to isolate and characterize endophytic actinobacteria from these plants, with a specific focus on evaluating their efficacy in inhibiting multidrug-resistant pathogens that pose a significant threat to human health.

REVIEW OF LITERATURE

Actinobacterial research is a rapidly growing field that includes various aspects of actinobacterial biology, including their diversity, ecology, physiology, genetics, and applications. some of the current research areas in actinobacterial research include discovery of novel bioactive compounds, antimicrobial resistance, Plant-microbe interactions, Environmental applications, Genomics and genetics and Synthetic biology. Researchers have been isolating actinobacteria from various sources, including soil from diverse ecosystems, such as forests, grasslands, and deserts, plant tissues including roots, stems, and leaves, marine sediments and coral reefs, and extreme environments such as hot springs, Antarctic soils, and deep-sea sediments, in search of new bioactive substances that could be utilized in medicine, agriculture, and biotechnology. These efforts have led to the discovery of new actinobacterial species and strains, which have been found to yield a wide range of secondary metabolites, such as antibiotics, antifungals, anticancer agents, and enzymes. actinobacteria were considered transitional forms between the fungi and bacteria (Barka et al., 2016).

The comparison between actinomycetes and fungi may appear valid at a glance, but it only scratches the surface. In reality, actinomycetes share a greater number of characteristics with bacteria than with fungi. Unlike the robust structures typically found in fungal cells, actinobacteria possess thin cell walls composed of peptidoglycan. Their genetic material is organized in a prokaryotic nucleoid, which is a key feature distinguishing them from more complex eukaryotic organisms. This unique cell structure underscores their bacterial similarities while highlighting the distinctive traits that set actinobacteria apart in the microbial world. Endophytic actinobacteria are remarkable microorganisms that live within the tissues of plants and are recognized for their ability to synthesize diverse bioactive chemical compounds. These compounds often feature unique structural characteristics and hold significant potential for medicinal applications (Gayathri and Muralikrishnan, 2013; Singh and Dubey, 2015). Numerous studies have highlighted the antimicrobial properties of these compounds, showing the vast potential of endophytic actinobacteria in the realm of natural product discovery. Among these, the genus Streptomyces is the most frequently isolated and studied group of endophytic actinobacteria, playing an

important role in the search for new antimicrobial agents (Zhao K. et al., 2011; Golinska et al., 2015).

Among the significant compounds of biological significance that have been isolated from various species of *Streptomyces* are munumbicins A and B, known for their potential therapeutic applications. Additionally, naphthomycins A and K have also gained attention due to their intriguing biological properties. Other noteworthy compounds include clethramycin, which is recognized for its antibiotic capabilities, and coronamycin, celebrated for its distinctive chemical structure and activity. Cedarmycins A and B, along with saadamycin and kakadumycin, further exemplify the wide range of bioactive substances derived from these remarkable actinobacteria. In a broader context, additional active compounds have been sourced from other actinobacteria, for instance, paclitaxel, an important anticancer agent, is extracted from *Kitasatospora* sp., which is notably associated with the yew tree, *Taxus baccata*. Another compound, tyrosol, has been identified in *Emblica officinalis* and is suggested to possess properties that inhibit food-borne microbes, highlighting its potential role in food safety and preservation (Zhao K. et al., 2011; Gangwar et al., 2014; Golinska et al., 2015).

History of endophytic actinobacteria

The intriguing history of endophytic actinobacteria can be traced back to the end of 19th century, specifically in 1866 when the pioneering German botanist Anton de Bary made a groundbreaking discovery. He identified the presence of microorganisms residing within the tissues of plants, opening a new frontier in our understanding of plant biology. However, it wasn't until the latter part of the 20th century that the scientific community began to research deeper into the potential benefits offered by these microorganisms, with a particular focus on actinobacteria. Throughout the 1990s and into the early 2000s, researchers embarked on a journey of exploration, isolating and characterizing endophytic actinobacteria from an array of plant species. This rigorous research revealed the remarkable fact that actinobacteria serve as a storehouse of bioactive compounds. These compounds include a variety of therapeutic agents such

as antibiotics, antifungals, and anticancer agents, highlighting the substantial promise these microorganisms hold in the realm of medicine.

Today, the study of endophytic actinobacteria remains a dynamic and evolving field. Researchers are actively investigating their potential applications across multiple domains, including the discovery of novel bioactive substances, understanding the complex interplay between plants and microorganisms, and exploring biotechnological applications that could revolutionize agriculture and medicine. The ongoing research into these fascinating organisms continues to show new insights and possibilities, contributing to both scientific knowledge and practical advancements.

Apart from the disease-causing microorganisms, the presence of other (non-pathogenic) organisms inside the plants was first pronounced by De Bary (1866), who detected the presence of microbial cells in the microscopically analysed plant tissues. This observation was not explored until the end of the last century when the term "endophytes" was defined. De Bary (1866) provided the first definition of an endophyte, as “any organism that grows within plant tissues are termed as endophytes,” however, the definition continues to change as per various researchers (Wilson, 1995; Hallmann et al., 1997; Bacon and White, 2000). Petrini (1991) provided the most suitable definition for endophytes, which means any organism that at some part of its life cycle, colonizes the internal plant tissues without causing any type of harm to the host plant. Furthermore, due to extensive studies of these groups of microorganisms, the endophytic communities have been divided into different subgroups, such as ‘obligate’ or ‘facultative,’ which are associated with all types of plants (Rosenblueth and Martínez Romero, 2006).

In recent years, a considerable number of studies have aimed to unravel the origins of endophytic organisms found in various plant species (Hallmann et al., 1997; Mitter et al., 2013). Researchers initially focused on two primary sources for these endophytes: the rhizosphere which is the region of soil that is surrounded by plant roots and microbial communities present in seeds. These environments serve as crucial reservoirs for the diverse array of endophytic microorganisms that interact with plants. The dynamics of these specific endophytes and their relationships with different plant

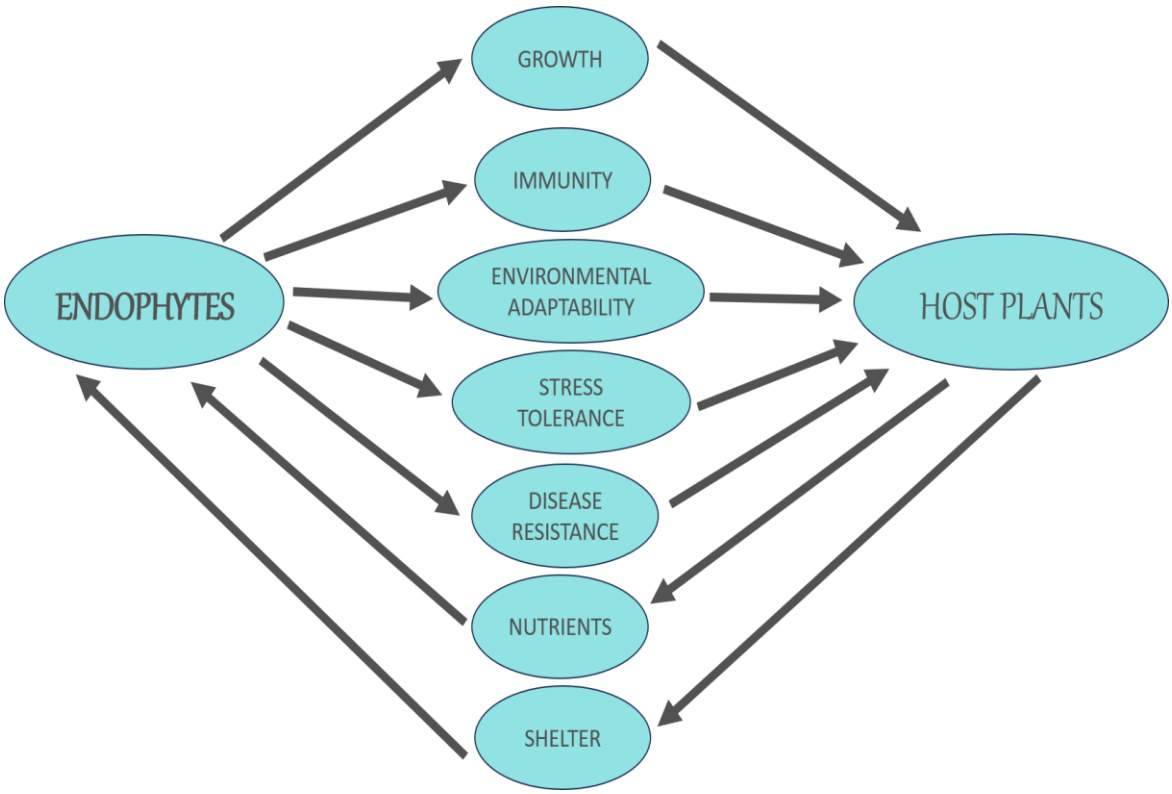
species are often reflected in their genomic structures. This genomic organization provides valuable insights into how endophytes adapt, thrive, and propagate within their host plants (Andreote et al., 2014). A number of scientists have precisely examined the sizes and origins of endophytes' genomes, linking genome size to the lifestyle strategies of these bacteria (Dini Andreote et al., 2012).

Endophytes are typically defined as organisms that inhabit plant tissues, wherein the environment is generally more constant compared to the fluctuations of their natural soil habitat. This notion supports the understanding that these microorganisms can successfully colonize and persist within plants. However, it's important to note that some endophytes only emerge within the plant during specific stages of their life cycle, indicating a more complex relationship. Consequently, the endophytic community is composed of organisms from diverse ecological backgrounds. Particularly those endophytes with larger genomes tend to thrive in variable environments, such as soil, while those with smaller genomes appear to be more adapted to the stable conditions inside the plant and are often transmitted vertically from one generation to the next (Mitter et al., 2013). In recent years, a considerable number of studies have aimed to discover the origins of endophytic organisms found in various plant species (Hallmann et al., 1997; Mitter et al., 2013).

Association of endophytes with host plants

The quality and product of medicinal plants are immensely affected by a variety of environmental factors. These include temperature, which can determine the rate of growth; illumination, which is essential for photosynthesis; moisture levels that affect hydration and nutrient uptake; soil conditions such as pH and fertility that influence plant health; and the presence and diversity of soil fauna that contribute to soil ecosystem dynamics (Namdar et al., 2019). Furthermore, recent studies have increasingly highlighted the significant role that specific bacterial endophytes (a group of beneficial microorganisms that inhabits the plant tissues) can play in shaping the growth and secondary metabolite production of medicinal plants (Ek-Ramos et al., 2019). These complex relationships between plants and their microbial companions

highlight the complexity of plant growth and the potential for enhancing medicinal properties through microbial interactions.



Long-term, symbiotic relationships between host plants and their associated endophytes can profoundly enhance plant growth and provide significant benefits for agricultural practices (Compant et al., 2010). These beneficial bacterial endophytes reside in various parts of the plant such as flowers, leaves, roots, seeds, and stems where they thrive and foster a dynamic partnership that is vital to the overall health and development of their host (Qin et al., 2009; Elmagzob et al., 2019). The presence of these microorganisms enriches the vitality of the plants by facilitating nutrient acquisition, strengthening resilience against environmental stressors, and enhancing disease resistance. This makes them invaluable allies in the pursuit of sustainable farming, as they not only support plant growth but also contribute to the ecological balance and health of agricultural ecosystems.

The intricate relationship between endophytes and their host plants is best characterized as symbiotic; this mutually beneficial association allows both organisms to thrive. The dynamic interaction between host plants and the diverse endophytic

community represents a co-evolutionary journey, shaped by a myriad of factors including the plant's genetic makeup, developmental stage, physiological health, and the specific type of plant tissue involved. Additionally, agricultural practices and environmental conditions, such as water availability, nutrient levels and temperature fluctuations, play crucial roles in the colonization process of these bacteria. A wealth of research highlights the profound influence host plants exert on their associated endophytic bacterial communities. For instance, a study conducted in the unique Patagonian ecosystem uncovered striking differences in both the abundance and diversity of bacterial populations associated with different plant species. It was found that *Hieracium pilosella* harboured a significantly greater variety of bacteria compared to *Gaultheria mucronata* (Zhang et al., 2019). This finding emphasizes the importance of the distinct physiological characteristics, biochemical metabolites, and growth patterns exhibited by different plant species, which ultimately enhance their ability to attract a wide array of endophytic bacteria Kawaguchi and Minamisawa, 2010; Campisano et al., 2014).

The health of host plants is an essential factor that significantly influences the composition of their endophytic communities. A notable example is found in *Paullinia cupana*, where plants exhibiting asymptomatic anthracnose are predominantly colonized by bacteria from the Firmicutes phylum. In stark contrast, those that are suffering from symptomatic anthracnose display a significant increase in the dominance of Acidobacteria within their endophytic community (Bogas et al., 2015). These observations suggest that the presence of pathogens may lead to visible surface damage on the host plant, creating opportunities for specific endophytes to flourish. Meanwhile, this disruption can unbalance the previously stable microbial ecosystem that existed within the plant. As highlighted by Hallmann et al. (1998), this transformation in microbial community dynamics highlights the complex and intricate relationship between plant health and microbial colonization. Such shifts not only affect the survival of the plants but also the diversity and functionality of the microorganisms that inhabit them, illustrating the profound interplay between biotic stressors and microbial life.

Endophytic actinobacteria are fascinating microorganisms that live within the inner tissues of plants without causing any harm. These beneficial bacteria have demonstrated impressive potential in promoting the growth of medicinal plants. They achieve this enhancement by significantly increasing both root and shoot biomass, which are vital for the overall vigour and health of the plant. Additionally, these endophytes play a crucial role in facilitating seed germination, as noted by Vendan et al. in their 2010 study. Research has shown that the endophytic actinobacteria has the ability of growth-promoting effects by having the capability to produce plant hormones, particularly indole acetic acid (IAA). This natural compound is essential for regulating a variety of physiological processes within plants, thereby contributing to improved growth and development, as highlighted by the findings of Fouda et al. in 2021. Through these mechanisms, endophytic bacteria offer a promising pathway for enhancing the cultivation and efficacy of medicinal plants.

Among the various endophytic bacteria, *Serratia marcescens* AL2-16 distinctly captures attention due to its remarkable ability to fix nitrogen within the plant species *Achyranthes aspera*. This intriguing process involves the bacteria harnessing atmospheric nitrogen and transforming it into a form that is biologically accessible to the plant, enabling it to thrive. Through a series of intricate enzymatic reactions, *Serratia marcescens* converts inert nitrogen from the air into essential nutrients that the plant can easily absorb and utilize. This nitrogen-fixing prowess plays a vital role in bolstering the nutritional support of *Achyranthes aspera*. As highlighted by Devi et al. (2016), the involvement of *Serratia marcescens* not only enhances the growth and development of this medicinal plant but also positions it as a crucial ally in fostering overall plant health and productivity. The partnership between this endophytic bacterium and its host plant exemplifies the symbiotic relationships that can significantly impact ecological and agricultural systems.

Certain endophytes play a significant role in enhancing the adaptability of their host plants against various abiotic stressors, such as heavy metals and salinity, as noted by Sheng et al. (2011). Salinity acts as a primary obstruction to plant growth by reducing the soil's osmotic potential. In response, plants are compelled to decrease their own water potential in a bid to absorb and retain sufficient water. In these harsh conditions,

the survival of host plants can be strengthened through mechanisms such as the modulation of phytohormones, which help alleviate the detrimental effects of osmotic stress (Hasanuzzaman et al., 2014). In one intriguing experiment, the endophytic bacterium *Achromobacter xylosoxidans* demonstrated its capacity to lower ethylene levels in the plant species *Catharanthus roseus*. At the same time, it significantly elevated the activity of various antioxidant enzymes, such as ascorbate peroxidase, catalase, and superoxide dismutase, all of which are essential for managing stress under saline conditions (Karthikeyan et al., 2012). Exploring the relationship between endophytic bacteria and the metabolism of their medicinal host plants presents numerous challenges, particularly due to the potential overlap in secondary metabolite production that can stem from the cooperative interactions between the bacteria and the host plant (Brader et al., 2014). Indeed, certain endophytic bacteria are well-documented for their ability to stimulate the biosynthesis of secondary metabolites in medicinal plants, amplifying their therapeutic properties (Tiwari et al., 2010). For example, the bacterium *Bacillus altitudinis* KX230132.1 is recognized as an effective elicitor that significantly boosts the levels of ginsenosides in the highly esteemed medicinal plant, ginseng (Song et al., 2017). Prior studies have even indicated that endophytes residing within host plants can synthesize a diverse range of bioactive secondary metabolites, including cyclopeptides, alkaloids, lactones, sesquiterpenes, flavonoids, polyketones, organic acids, and saponins. These compounds hold immense potential for novel applications in various fields (Ek-Ramos et al., 2019). Furthermore, additional investigations into the activities of these endophytic actinobacteria and their host plants have revealed a wealth of shared bio-properties. These include a range of therapeutic effects such as antimicrobial, anticancer, anti-inflammatory, and anti-HIV activities (Rustamova et al., 2020). Such findings highlight the deep impact that endophytic actinobacteria can have on enhancing the medicinal value and overall stress resilience of their host plants.

Antibiotics produced by Streptomyces

Streptomyces are remarkable actinobacteria known for the original source of a various number of the world's antibiotics. These fascinating microorganisms belong to the phylum Actinomycetota. Unlike motile bacteria, Streptomyces are non-motile and

exhibit a unique method of spreading through the production of threadlike structures called hyphae. These hyphae penetrate their surroundings in search of essential nutrients, allowing the bacteria to thrive in various environments. In times of nutrient scarcity, Streptomyces display a remarkable survival strategy. They generate aerial hyphae that rise above the substrate and subsequently undergo division to form spores. These spores are resilient, designed to withstand harsh and unfavourable conditions. Additionally, their lightweight nature facilitates easy dispersal to new environments, enabling Streptomyces to colonize fresh sources of nutrients effectively. This ability to adapt and proliferate highlights the ecological significance and versatility of these extraordinary actinobacteria. (Chater 2016). During World War II, the pressing need for effective antibiotics became critical, especially as penicillin proved inadequate for treating Tuberculosis (TB) and certain Gram-negative bacterial infections. It was in this atmosphere of urgency that the microbiologist Selman Waksman and his team, including the researcher Albert Schatz, embarked on a quest to discover new antimicrobial agents. Their groundbreaking work led to the isolation of an extraordinary substance called streptomycin. This antibiotic was derived from a strain of the bacterium *Streptomyces griseus*, which was found thriving in richly manured compost soil. In a remarkable twist of fate, another sample contributing to this discovery came from the digestive tract of a chicken, illustrating the diverse environments from which life-saving medications could emerge. (Wainwright, 1991). In the 1980s, researchers from the Merck group made a significant breakthrough by discovering platensimycin, a novel class of antibiotic derived from the bacterium *Streptomyces platensis*. This innovative antibiotic selectively targets and inhibits the biosynthesis of cellular lipids, which are essential components of cell membranes. The unique mechanism of action of platensimycin positions it as a promising candidate in the fight against antibiotic resistance, highlighting its potential for therapeutic applications in treating bacterial infections (Martens and Demain, 2011; Wang et al., 2006). In recent years, numerous antibiotic molecules have been discovered from the genus Streptomyces, particularly those isolated from traditional medicinal plants across the globe. For instance, Cycloheximide, actiphenol, and diketopiperazine have been successfully extracted from endophytic Streptomyces strains found in *Arnica montana* L., as reported by Conti et al. in 2016. Another remarkable finding includes

Vinaceuline cyclodipeptides, which were derived from endophytic *Streptomyces* sp. associated with *Paraboea sinensis*, highlighting the diverse ecological relationships these bacteria form with their plant hosts (Yang et al., 2013). Furthermore, Pluramycin was isolated from endophytic *Streptomyces* sp. residing in the roots of *Heracleum souliei*, demonstrating the potential of these microbes to produce novel antibiotic compounds (Liu et al., 2014). A particularly noteworthy discovery is Brevianamide F and the cyclo-(l-Pro-l-Phe) compound, both of which exhibit effectiveness against methicillin-resistant *Staphylococcus aureus* (MRSA). These compounds were isolated from an endophytic Streptomyces inhabitant of *Vochysia divergens* in Brazil, as detailed by Gos et al. in 2017. Such findings highlight the rich symbiotic relationships between endophytic bacteria and their host plants, serving as a promising source for new antibiotics in the ongoing fight against antibiotic resistance.

Epidemiology of MRSA

Hospital-acquired MRSA (HA-MRSA) has become one of the most challenging bacterial infections to manage (Kaur and Chate, 2015) and disturbingly, accounts for approximately 20–80% of infections in hospitals (Krishnamurthy et al., 2014). Although a 54.2% decrease in the occurrence of HA-MRSA has been reported in the USA (Dantes et al., 2013), data from other regions of the world show that this is not a common pattern. For instance, research conducted in South Africa, India, and Pakistan found that MRSA accounted for 52%, 54.8%, and 50% of hospital-acquired infections, respectively (Laxminarayan et al., 2013). In hospital environments, Methicillin-resistant *Staphylococcus aureus* (MRSA) frequently manifests as a secondary infection, predominantly affecting vulnerable populations such as the elderly, post-surgical patients, and those with compromised immune systems (Krishnamurthy et al., 2014). The emergence of these secondary infections not only poses serious health risks but also worsens the financial burdens on healthcare systems, leading to longer hospital stays and the necessity for additional antibiotic treatments (Nelson et al., 2015).

In healthcare environments, the challenge of managing the transmission of Methicillin-resistant *Staphylococcus aureus* (MRSA) is significantly worsened by its capability to

form biofilms on the surface of different medical appliances. These biofilms not only display remarkable resistance to standard disinfectants but also act as persistent reservoirs for MRSA colonies, presenting a risk of contamination and transmission to other patients and healthcare workers (Suzuki et al., 2015). Research indicates that the carriage rate of MRSA in hospitals can range from 20% to 60% (Pathare et al., 2016), highlighting a concerning prevalence among both healthcare professionals (El Aila et al., 2017) and patients (Moyo et al., 2017). Typically, MRSA gains entry into a host through breaks in the skin, such as cuts or surgical incisions (Datta et al., 2014), and often leads to infection when the host's immune defences are compromised. This pathogen poses a particularly severe threat to vulnerable patient populations, complicating treatment protocols and inflating healthcare costs. While individuals with weakened immune systems are at heightened risk for MRSA infections, there has been a troubling emergence of cases among healthy individuals, particularly in children (Davoodabadi et al., 2016). This shift underscores the evolving nature of MRSA and the urgent need for enhanced preventive measures in healthcare settings to safeguard both high-risk and healthy patients alike.

In Japan, a recent in-depth comparative cost analysis of Methicillin-resistant *Staphylococcus aureus* (MRSA) has revealed that its financial burden far exceeds that of all other non-MRSA infections (Uematsu et al., 2017). This stark statistic underscores the formidable threat posed by these resilient organisms. In the United States, MRSA has become a grave public health concern, claiming more lives than the combined total of HIV and tuberculosis (Boucher and Corey, 2008). Such alarming figures highlight the urgent requirement for effective prevention and treatment strategies to fight this pervasive infection.

Isolation of Endophytic Actinobacteria

Researchers utilize numerous techniques to effectively isolate endophytic actinobacteria, and the diversity observed among actinobacteria is significantly influenced by the specific isolation method chosen. In their 2006 review, Hallman et al. thoroughly analyse and discuss numerous protocols for the isolation of these microorganisms, providing valuable insights into their effectiveness and applicability.

Meanwhile, Coombs and Franco (2003) have contributed to this field by introducing several innovative isolation methods, highlighting the ongoing advancements in the understanding of these fascinating microbial communities.

The selected plants are sourced from a variety of locations, ensuring a diverse genetic pool. Once gathered, they are kept under strictly sterile conditions to preserve their integrity until they are ready for use. A critical stage in the preparation process involves surface sterilization of the explants, a procedure designed to eradicate any unwanted microorganisms that may be clinging to the plant surfaces. To achieve effective sterilization, several commonly used agents are employed, including ethanol in concentrations ranging from 70% to 95%, sodium hypochlorite at 3% to 10%, and hydrogen peroxide, as noted by Gilbert et al. (2023). After the sterilization process, each explant undergoes a thorough rinsing with distilled water to remove any residual chemicals. To further combat the risk of fungal growth, which could potentially overshadow the actinobacteria in later stages, the plant tissues are sometimes immersed in a 10% sodium bicarbonate (NaHCO3) solution, as suggested by Poschenrieder et al. (2018). This additional step serves as a precautionary measure, promoting a healthier environment for the subsequent cultivation of these valuable plant tissues.

The subsequent phase of the procedure centres on the vital pretreatment of plant tissue, which is essential for successful bacterial isolation. Initially, any bacteria residing on the surface of sterilized plant samples are eradicated by subjecting the tissues to high temperatures ranging from 80 °C to 100 °C for a duration of 15 to 30 minutes. This exposure effectively eliminates surface contaminants, ensuring a cleaner starting point for further analysis. (Sahu et al., 2022). Following this thermal treatment, the explants are cut into smaller segments, which are then introduced into specially prepared culture media designed to foster bacterial growth. Alternatively, the plant tissues can undergo a more aggressive processing method, utilizing a blender for homogenization or being crushed into a fine mixture with a mortar and pestle. During this process, the tissues are often mixed with a phosphate buffer or a tailored extraction solution, as discussed by Schulz & Boyle, 2006, to facilitate the release of intracellular components. To inhibit the growth of fungi during the culturing process, antifungal such as nystatin

and cycloheximide are incorporated into the media. This specialized media is then incubated at a controlled temperature of 26 ± 2°C for a duration ranging from 15 to 30 days. After this incubation, colonies of actinobacteria isolates, each displaying unique and distinct morphological characteristics, are carefully selected for further study. To ensure the purity of these isolates, the process of repeated streaking is employed on fresh nutrient media plates, allowing for the establishment of pure cultures necessary for subsequent experiments (Bonnet et al., 2019).

The growth of actinobacteria is profoundly influenced by both the composition of the culture media and the specific conditions under which they are cultivated. Various researchers have put forth a range of growth media geared toward the successful isolation of endophytic actinobacteria, each tailored to optimize their development. For instance, Kuster and Williams (1964) introduced a starch casein media, which provides essential nutrients for the growth of these microbes. Similarly, Meenakshi et al. (2024) proposed the use of ISP7, also known as Tyrosine Agar, specifically designed to encourage the proliferation of actinobacteria. The starch casein nitrate (SCNA) medium, recommended by Passari et al. (2015), is another option that combines starch and nitrogen sources to support microbial growth effectively. Hayakawa (2008) suggested a chitin vitamin B medium, which not only fosters growth but also provides vital vitamins that can enhance the metabolic activities of these microorganisms. The tap water yeast extract (TWYE) medium recommended by Crawford et al. (1993) blends yeast extract with tap water, creating an environment favourable to actinobacteria. Furthermore, humic acid vitamin B (HV) media and yeast extract casamino acid (YECA) media, as mentioned by Mincer et al. (2002), introduce different organic compounds that can stimulate growth through various nutritional pathways. Lastly, Kuster (1959) brought forth the glycine-glycerol medium, which is essential for promoting the growth of specific actinomycete strains through the incorporation of glycerol as a carbon source. Each of these media formulations underscores the significance of tailored nutrient environments in the effective cultivation of actinobacteria.

Molecular identification of Actinobacteria

The molecular characterization of actinobacteria is a detailed and intricate process that focuses on identifying these organisms at the genetic level. This approach is widely recognized as one of the most consistent and precise methods for pinpointing and classifying organisms down to the species level. The first step in this process involves isolating DNA from pure cultures of actinobacteria, ensuring that we have a clean genetic sample. Following this, specific genes of interest are amplified using the polymerase chain reaction (PCR), a reliable technique that allows researchers to make millions of copies of a targeted DNA segment. Once the amplification is complete, the next crucial step is sequencing the amplified genes, which enables scientists to determine the genetic makeup of the organism. This comprehensive methodology not only aids in the identification of actinobacteria but also enhances our understanding of their diversity and evolutionary relationships.

The process of DNA isolation is critical for the successful extraction of genomic DNA from actinobacteria, and it necessitates a standardized protocol to ensure consistency and reliability. Over the years, numerous procedures have been developed by researchers around the globe, each aiming to optimize the extraction of high-quality DNA. Additionally, a variety of commercial isolation kits have emerged, making the process more accessible to laboratories. Among the most widely utilized genomic DNA isolation kits are the PureLink® Genomic DNA Kit from Invitrogen™, the FastPrep Kit manufactured by MP Biomedicals in the USA, and the PowerSoil® DNA Isolation Kit, as noted in the research conducted by Shaikh et al. in 2015. Each of these kits offers unique features and benefits, catering to the diverse needs of researchers working with actinobacteria, ultimately facilitating more efficient and effective DNA isolation practices.

The 16S ribosomal RNA (rRNA) gene and the Gyrase B gene are among the genetic markers most frequently utilized for the molecular identification of actinobacteria, a diverse category of bacteria recognized for their significant roles in medicine and ecology. The 16S rRNA gene stands out as the primary housekeeping genetic marker due to several compelling reasons. Firstly, this gene is universally present in nearly all

bacterial species, often organized in a multigene family or within operons, allowing for a broader range of analysis across different taxa. Secondly, the functional consistency of the 16S rRNA gene throughout evolutionary history is notable; its relatively stable sequence over time permits random variations to serve as reliable indicators of evolutionary relationships. This stability, combined with the gene's length approximately 1,500 base pairs make it particularly well-suited for computational analysis and bioinformatics applications. By utilizing universal primers to amplify the 16S rRNA gene, researchers can generate sequences that enable detailed analysis of phylogenetic relationships among bacteria. However, to ensure the accuracy and reliability of these analyses, researchers must address several significant challenges. Factors such as primer bias, contamination of samples, and the quality of the resulting sequences can all impact the integrity of the data. To combat these issues, best practices in molecular biology recommend a various approach employing various primers to mitigate bias, incorporating negative controls to detect contamination, confirming the quality of sequences produced, and utilizing robust bioinformatics tools for analysis. These practices are essential for producing reliable results that further our understanding of actinobacteria and their roles in various environments.

Antimicrobial potential of *Streptomyces* sp.

A primary metabolite refers to a class of metabolites essential for the fundamental processes of growth, development, and reproduction in living organisms. These compounds play critical physiological roles, ensuring the proper functioning of cellular activities. Primary metabolites are commonly found across a wide range of organisms and cells, demonstrating their fundamental importance in biological systems (Demain 1980). In contrast, secondary metabolites are organic compounds that are not involved directly in the essential functions of growth or reproduction. Instead, they often serve specialized roles in organisms that can provide advantages in their environment, such as defence mechanisms or interactions with other species (Shomurat et al., 1979).

One of the most notable characteristics of Streptomyces, a genus of bacteria within the actinobacteria phylum, is their remarkable ability to produce a vast range of secondary

metabolites. Many of these compounds exhibit potent properties, including antibacterial, antifungal, antiviral, and antitumoral effects. This capacity for secondary metabolite production is a defining feature of Streptomyces, which is responsible for producing over 80% of the total antibiotic products derived from actinobacteria. In comparison, other less common actinobacteria, such as *Actinopolyspora* and *Micromonospora*, contribute a fraction of this antibiotic output, amounting to less than one-tenth of the Streptomyces population. Moreover, it has been estimated that approximately 42% of commercially available metabolites originate from various actinomycetes, while around 16% are derived from fungal sources, with the remainder coming from other bacterial sources (Al-Ansari et al., 2019). This shows the significant role that actinobacteria play in the production of valuable metabolites in both natural and industrial contexts.

Endophytic actinobacteria, particularly those isolated from medicinal plants, have gathered significant attention from researchers due to their remarkable ability to produce bioactive compounds that can effectively inhibit the growth of various microorganisms. For instance, a study by Castillo et al. (2006) highlighted the isolation of two chromophoric peptide antibiotics, munumbicins E-4 and E-5, from the endophytic organism *Streptomyces* NRRL 30562. In addition to these two compounds, this particular strain was also found to produce a series of broad-spectrum antibiotics known as munumbicins A through D. Remarkably, both munumbicins E-4 and E-5 demonstrated strong broad-spectrum antimicrobial activity, showing effectiveness against both gram-positive and gram-negative bacterial strains. Further research conducted by Zhang et al. (2012) identified a selection of twelve strains of endophytic actinomycetes, belonging to the genera Streptomyces and Glycomyces, which were isolated from various medicinal plants, including *Achyranthes bidentata*, *Paeonia lactiflora*, *Radix platycodi*, and *Artemisia argyi*. These strains exhibited significant inhibitory effects on penicillin-resistant *Staphylococcus aureus*, highlighting their potential as a source of novel antibiotics and their importance in the ongoing fight against antibiotic resistance. El-Gendy et al. (2018) reported fascinating findings regarding two endophytic strains of Streptomyces, specifically *Streptomyces* sp. RS-9 and *Streptomyces* sp. MS-26. These strains exhibited significant antagonistic activity

against 18 different isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), highlighting their potential as sources for novel antibacterial agents. Additionally, it is noteworthy that daptomycin, an antibiotic derived from Streptomyces, is the only naturally produced antibiotic of a new class that has been introduced since 2003. This antibiotic is now recognized as a first-line treatment for MRSA bacteria, as noted by Choo and Chambers (2016), underscoring its vital role in combating this challenging and resilient strain of bacteria.

Streptomyces, a genus known for its remarkable diversity, harbours an extensive genome that logically encompasses a multitude of biosynthetic gene clusters. These clusters are crucial as they hint at the organism's potential to synthesize a vast array of compounds, each with unique and varied biological activities (Bentley et al., 2002; Ikeda et al., 2003). However, the reality of conventional culture conditions reveals a stark contrast; only a fraction of the compounds predicted by the genome have been successfully isolated. This discrepancy highlights the limitations of traditional methods in unlocking the full biosynthetic potential of Streptomyces.

In response to this challenge, researchers are increasingly turning to modern genetic engineering techniques. These advanced tools enable scientists to identify and target specific gene clusters that are linked to potentially valuable compounds. Once these biosynthetic gene clusters are located, they offer a wealth of opportunities for modification. Scientists can enhance the effectiveness of the compounds, design superior analogues, or significantly increase the yield of the desired products (Alexander et al., 2010; Yang et al., 2017). Additionally, innovative in situ computer models have proven to be valuable in discovering the mechanisms of action behind certain promising anti-MRSA compounds derived from Streptomyces. These models facilitate a deeper understanding of how these compounds function at a molecular level, paving the way for the development of new therapeutics against resistant strains of bacteria.

Antioxidant potential of *Streptomyces* sp

Life on Earth is intricately tied to the presence of oxygen, a vital element that sustains countless forms of life. However, this same oxygen can also present significant

dangers, particularly through the phenomenon of oxidative stress, which occurs when reactive oxygen species (ROS) are generated within cells and tissues. These ROS, while a natural byproduct of various metabolic processes, can lead to cellular damage if not adequately managed. Antioxidants play a crucial role in maintaining cellular health by acting as scavengers that seek out and neutralize these harmful ROS. They protect vital entities and molecules from oxidative injury through various mechanisms of action. Antioxidants can generally be divided into two categories based on how they function: primary and secondary antioxidants. Primary antioxidants are particularly noteworthy for their ability to combat free radicals through two distinct strategies. One approach is known as hydrogen atom transfer (HAT), wherein these antioxidants donate an H-atom to free radicals, effectively neutralizing them. The second method, referred to as single electron transfer (SET), involves the transfer of an electron to the free radical, disrupting its harmful activity. Remarkably, primary antioxidants can neutralize a substantial number of free radicals even when present in relatively small quantities. A prime example of these powerful compounds is phenolic antioxidants, which are celebrated for their remarkable catalytic properties. They not only provide protection against oxidative stress but can also be readily regenerated, allowing them to continue their protective role effectively (Zeb 2020). This remarkable ability highlights the importance of antioxidants in safeguarding cellular integrity and promoting overall health.

Mycothiol (MSH) is an essential thiol compound, often referred to as a “sugar” thiol, that is predominantly found in the cell walls of actinomycetes, a group of bacteria known for their complex structures and ecological importance. Serving as a functional counterpart to glutathione (GSH), MSH takes on a critical role in the cellular machinery of these organisms. Notably, actinobacteria lack the enzymes necessary for GSH synthesis, leading them to rely on alternative low molecular weight thiols (LMW) as substitutes. Among these alternatives, bacillithiol (BSH) and ergothioneine (ESH) are noteworthy, but MSH stands out as a primary example. The importance of MSH cannot be overstated, as it plays an important role in maintaining intracellular redox homeostasis, a key process that ensures the proper functioning of various biological mechanisms vital for the health and viability of cells. These mechanisms encompass

crucial activities such as the activation of enzymes, DNA synthesis, and the regulation of the cell cycle, all of which are essential for cellular integrity and function. Functioning as a powerful antioxidant, MSH has the remarkable ability to both donate and accept electrons. This characteristic enables it to act as a crucial cofactor in detoxifying a wide array of harmful substances, including free radicals, xenobiotics, and alkylating agents that can pose threats to cellular stability. The structural composition of MSH further distinguishes it from GSH. While GSH consists of two amino acids—glycine and glutamic acid—MSH is uniquely characterized by its two sugar components: N-glucosamine and inositol, supplemented by a cysteine unit. This distinctive structure not only sets MSH apart from GSH but also highlights its specialized function within the biological framework of actinobacteria, underscoring its pivotal role in their survival and adaptation to challenges in their environment (Quach 2020).

Chandra et al. (2020) conducted a comprehensive investigation into the various reactive functional groups present in various natural compounds that contribute to the remarkable antioxidant properties of terpenoids, isoflavonoids, and specific derivatives such as 4', 7, 8-Trihydroxyisoflavone, as well as unique metabolites like naphterpins B and C, carazostatin, and carbazomycin B, which are all derived from the bacterial genus Streptomyces. A notable discovery from this research is the identification of phenazoviridin, the first glycosylated phenazine derivative isolated from Streptomyces. This compound has emerged as a highly effective inhibitor of lipid peroxidation, demonstrating superior protective effects against acute hypoxia induced by potassium cyanide (KCN) in mice, especially when compared to the commonly used drug indeloxazine (Kato et al., 1993). Further enhancing our understanding of natural antioxidants, Thiazostatins A and B, along with a series of benzastatins (A to D) produced by *S. tolurosus* and *S. nitrosporeus*, illustrate the diverse biochemical strategies employed by these organisms. These compounds act as nitrogen-holding antioxidants and feature distinctive structural components, such as a tetrahydroquinolone ring or a p-aminobenzamide unit. The benzastatin structure, characterized by an N-H group and a hydroxyl group, is further modified in its derivatives to include a methoxy group. Remarkably, the presence of these functional

groups enables these compounds to exhibit lower levels of lipid peroxidation compared to vitamin E (Guimarães et al., 2020; Shindo and Misawa, 2014). In addition to these findings, a range of compounds originating from Streptomyces, including antiostatins A1 to A4 and B1 to B4 isolated from *S. cyaneus*, as well as carbazoquinocins A to F derived from *S. violaceus*, showcase the presence of a carbazole framework with an o-quinone structure, further underscoring the significance of these natural antioxidants. Furthermore, neocarazostatins A to C, extracted from the mycelium of various Streptomyces strains, have been confirmed to possess distinct antioxidant properties (Kobayashi and Kuzuyama, 2020). This highlights the potential of carbazole derivatives from Streptomyces as a significant class of antioxidants. Moreover, Tan et al. (2019) provided compelling evidence for the antioxidant capabilities of specific strains of Streptomyces, namely *Streptomyces* sp. MUM212 and *Streptomyce*s sp. MUM265. Their research concluded that both strains are rich in phenolic compounds, which play a crucial role in inhibiting lipid peroxidation and reducing reactive oxygen species (ROS) through mechanisms that involve hydrogen donation and electron transfer. Additionally, Praptiwi et al. (2019) successfully identified organofluorine, a highly effective antioxidant, from *Streptomyces* sp. strain TC1, further expanding the array of natural antioxidants sourced from these remarkable bacteria.

Gas Chromatography Mass Spectrometry (GCMS)

Chromatography is a sophisticated separation technique that involves a mobile phase transporting a mixture that comes into contact with a stationary phase, which selectively adsorbs various components of the mixture. Among the numerous chromatography methods, Gas Chromatography (GC) stands out as a powerful tool for the separation and analysis of complex, multi-component mixtures, including essential oils, hydrocarbons, and various solvents.

In the process of gas chromatography, particularly the variant known as gas-liquid chromatography, the sample is first vaporized and then injected at the top of a chromatographic column. As the sample travels down the column, it is propelled by an inert gaseous mobile phase that flows continuously. The column itself is carefully

designed, containing a liquid stationary phase that is adhered to the surface of an inert solid substrate. This unique configuration allows for the effective separation of components based on the principles of adsorption and partitioning, where different substances interact differently with the stationary phase.

Gas chromatography is recognized as one of the most widely employed techniques within the chromatography field, owing to its efficiency and precision. Among its various applications, Gas Chromatography coupled with Mass Spectrometry (GC-MS) has become an invaluable tool for the detection and monitoring of organic pollutants in the environment. This advanced analytical technique is particularly useful for analyzing a range of compounds such as esters, fatty acids, alcohols, aldehydes, terpenes, and many others, providing vital insights into chemical compositions and environmental health (Al-Rubaye et al., 2017).

The gas chromatography-mass spectrometry (GC-MS) analysis of the extract derived from *Streptomyces albidoflavus* PU23 has revealed several noteworthy compounds, specifically 2,6-Lutidine and 3,5-dichloro-4-dodecylthio, alongside 2,5-Piperazinedione, 3,6-bis(2-methylpropyl). These compounds are likely responsible for the extract's notable antimicrobial properties, as suggested by the research conducted by Nandhini et al. in 2018.

In a separate investigation, the GC-MS analysis performed by Al-Rubaye et al. in 2020 on the crude extract of *Streptomyces* sp. uncovered the presence of Hexanoic acid, specifically 2-methyl-, which possesses the molecular formula C7H14O2 and a molecular weight of 103. This compound is recognized as a crucial element of the muramycin antibiotic, showcasing its potential significance in pharmaceutical applications.

Further dissecting the extract’s constituents, the GC-MS findings identified several predominant compounds, including pyrrolo[1,2-a]pyrazine-1,4-dione with a striking 36.85% concentration, followed by benzeneacetamide at 23.76%, and deferoxamine at 12.85%. Each of these compounds is well-documented for their pharmaceutical properties, highlighting the medicinal potential of the *Streptomyces* sp. extract as highlighted in the study by Tangjitjaroenkun et al. in 2021 regarding *S. achromogenes*

TCH4. The analysis conducted using Gas Chromatography-Mass Spectrometry (GC-MS) revealed a distinct chromatographic profile characterized by 16 prominent peaks. These peaks indicated the presence of various bioactive compounds within the sample. Among the noteworthy organic substances identified were 9-(2',2'-dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine, which presented a peak at 23.1 minutes, and Dotriacontylpentafluoropropionate, peaking at 25.0 minutes. Additionally, Octadecanoic acid was observed at 20.0 minutes, while Trans-2-methyl-4-n-butylthiane, S,S-dioxide appeared with a peak at 19.0 minutes. These findings underscore the significant potential of the ethyl acetate extract derived from *S. parvulus* VITJS11 in combating microbial infections, highlighting its broad range of efficacy and promising therapeutic properties (Naine et al., 2014).

High Performance Thin Layer Chromatography (HPTLC)

High Performance Thin Layer Chromatography (HPTLC) represents an advanced and highly efficient evolution of traditional Thin Layer Chromatography (TLC). This sophisticated technique is characterized by chromatographic layers that exhibit exceptional separation capabilities. HPTLC employs a range of advanced instrumentation designed to enhance every phase of the analytical process, including precise sample application and the development of standardized, reproducible chromatograms. The entire procedure is further refined through the use of software that controls and evaluates the results (Mamatha et al., 2011). HPTLC is built upon a solid foundation of widely standardized methodologies in scientific principles, complemented by the application of immensely validated methods for both qualitative and quantitative analyses. This approach ensures that HPTLC meets the stringent quality demands of contemporary analytical laboratories, thereby facilitating the achievement of higher resolution and more accurate quantitative measurements, ultimately benefiting various fields of research and quality control (Miyadoh et al., 1993).

A detailed investigation conducted by Shekar et al. in 2016 focused on the High-Performance Thin-Layer Chromatography (HPTLC) profiling of the ethyl acetate

extract derived from the *Streptomyces* sp. strain SFA5. The study successfully identified the presence of various antimicrobial compounds, which were observed in both the chromatographic results and under ultraviolet light following derivatization. To achieve optimal results, the researchers employed a well-defined solvent system for the mobile phase, comprising a mixture of toluene, ethyl acetate, methanol, and acetic acid in the ratio of 5:3:1:0.5 (v/v/v/v). In their analysis, the *Streptomyces* sp. SFA5 exhibited nearly 14 distinct peaks, each corresponding to different compounds, with notable retention factor (Rf) and area percentage values. Specifically, peak 7 displayed an Rf value of 0.4 with an area percentage of 29%, while peak 8 had an Rf value of 0.5 and an area percentage of 18%. These findings indicate a significant presence of antimicrobial substances, as evidenced by the distinct fingerprint profile of the ethyl acetate extract when analysed at UV wavelengths of 254 nm and 366 nm.

CHAPTER 2

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

- Isolation and identification of endophytic actinobacteria from selected medicinal plants of Mizoram forests.
- Antimicrobial screening of endophytic actinobacteria against clinical multidrug-resistant (MDR) clinical bacterial pathogens.
- Extract preparation, purification and characterization of antimicrobial compounds from selected potential isolates.
- Validation of purified bioactive compounds for their antimicrobial potency against MDR clinical pathogens.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. **Selection and collection of medicinal plants**

Five traditional medicinal plants from Mizoram were selected based on a literature review namely *Mikania micrantha* K, *Centella asiatica* (L.) Urb., *Tithonia diversifolia* (Hemsl), *Mirabilis jalapa* L., and *Clerodendrum colebrookianum* Walp. Samples of *Centella asiatica*, *Mikania micrantha* and *Mirabilis jalapa* were collected from Mizoram University (23°44'15.4"N 92°39'47.6"E) in Aizawl, while *Clerodendrum colebrookianum* Walp. were gathered from an agricultural farm in Tanhril (23°44'32.4"N 92°40'36.2"E), Aizawl. *Tithonia diversifolia* was collected from outside Mizoram University Campus roadside (23°44'28.0"N 92°40'37.0"E) in Tanhril. These plants were selected based on their antimicrobial activity and the relative abundance in the area. The various parts of the plants were collected in containers and brought back to the laboratory in sterile condition. They were thoroughly rinsed with tap water and it was air-dried in room temperature overnight.

3.2. **Isolation of Endophytic Actinobacteria**

Endophytic actinobacteria were isolated from five selected medicinal plants: *Mikania micrantha* K, *Centella asiatica* (L.) Urb., *Tithonia diversifolia* (Hemsl), *Mirabilis jalapa* L., and *Clerodendrum colebrookianum* Walp. Tissues from the roots, leaves, and stems of these plants were used for the isolation of actinobacteria. For surface sterilization, the tissues were rinsed in a 95% ethanol solution for 30 seconds, and it was rinsed with sodium hypochlorite solution (2% available Cl^-) for around 5 minutes. Finally, the tissues were washed three times with sterilized double-distilled water and dried in a laminar airflow cabinet (Taechowisan and Lumyong, 2003). Five different nutritional media were used for isolation to obtain maximum diversity i.e. Starch Casein Agar (SCA), Actinomycetes Isolation Agar (AIA), Tyrosine agar medium (ISP7), Glycerol asparagine agar base (ISP5), and King's medium. The whole plant was cut into small pieces (1.0 × 0.5 cm) for surface sterilization using sodium hypochlorite and 70% ethanol (Zin et al., 2007). Four to five pieces of each plant were then placed in media containing nalidixic acid and cycloheximide (50 μg/mL) to inhibit the growth of fungi and Gram-negative bacteria. The dried tissues were kept in

the media, and the plates were kept for incubation at 28±2°C in a BOD incubator. Microbial growth was observed, and actinobacteria were morphologically selected (Shirling and Gottlieb, 1966; Goodfellow and Haynes, 1984).

3.3. **Collection of Clinical MDR pathogens**

Clinical pathogens were systematically collected from patients at Synod Hospital, located in Durtlang, Aizawl, Mizoram. To ensure patient confidentiality, a numbering system was employed to label the collected pathogens. Each clinical isolate subjected to a process of identification using the Vitek®MS system (bioMérieux, Marcy l'Etoile, France), specifically utilizing the GP colorimetric identification card to determine their precise nature. Additionally, the antibiotic susceptibility of these pathogens was assessed through the Vitek®MS AST system, again from bioMérieux. A comprehensive list of the clinical pathogens identified during this study is presented in Table 3.1.

Table 3.1: Test organisms (MDR pathogens) used for the antimicrobial screening

S.No	**Pathogens**	**Organisms**	**Resistance**
1.	17	*Escherichia coli*	Meropenem, Ciprofloxacin, Trimethoprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem
2.	31	*Escherichia coli*	Meropenem,Amikacin, Gentamicin, Ciprofloxacin,Trimethprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem
3.	038	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

4.	161	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
5.	363	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
6.	365	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
7.	452	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
8.	536	*Enterococcus faecalis*	Vancomycin, Benzylpenicillin, Gentamicin, Daptomycin, Teicoplanin, Tetracycline, Ciprofloxacin, Erythromycin, Levofloxacin
9.	622	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
10.	645	*Staphylococcus aureus*	Rifampicin, Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
11.	721	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Erythromycin,

12.	790	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
13.	809	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
14.	880	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
15.	924	*Staphylococcus aureus*	Rifampicin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
16.	995	*Klebsiella pneumoniae*	Meropenem, Gentamicin, Ciprofolxacin, Tigecycline, Trimethoprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem,
17.	1097	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
18.	1098	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
19.	1219	*Enterococcus faecalis*	Vancomycin, Tetracycline, Erythromicin

20.	1246	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
21.	1258	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Benzylpenicillin, Ciprofloxacin, Erythromycin, Levofloxacin Vancomycin, Tetracycline
22.	1266	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
23.	1344	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
24.	1379	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
25.	1458	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
26.	1468	*Staphylococcus aureus*	Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
27.	1536	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Tetracycline, Levofloxacin
28.	2822	*Staphylococcus aureus*	Teicoplanin, Vancomycin, Tetracycline, Trimethoprim, Benzylpenicillin, Oxacillin, Erythromycin

29.	2982	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
30.	2997	*Escherichia coli*	Meropenem, Gentamicin, Ciprofolxacin, Trimethoprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem, Amikacin
31.	3001	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
32.	3054	*Pseudomonas aeruginosa*	Gentamicin, Cyprofloxacin, Levofloxacin, Colistin, Cefeazidime, Aztreonam, Imipenem, Meropenem, Amikacin
33.	3069	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,

3.4. **Primary antimicrobial screening of Endophytic Actinobacteria**

Actinobacteria isolates were subjected to testing for their ability to produce antimicrobial compounds against various clinical pathogens, as detailed in Table 1. To initiate the process, these isolates were grown in tryptone yeast extract broth (ISP1) media, placed in a shaker incubator set to 150 rpm, and allowed to grow for a period of three days. Following this incubation, the broth culture was centrifuged to separate the cells from the liquid, and the supernatant was carefully collected for further analysis. For the primary antimicrobial screening, the agar well diffusion method given by Saadoun and Muhana in 2008 was used. In this method, wells with a precise diameter of 6 mm were created on the surface of nutrient agar plates using a sterile

cork borer. After this preparation, the test pathogens were uniformly swabbed across the agar surface to ensure even distribution. A measured volume of 100 µl of the collected supernatant was then added into the pre-formed wells. To allow for the proper diffusion of the antimicrobial compounds into the agar medium, the plates were left undisturbed for several minutes. Subsequently, they were incubated at a temperature of 28°C for 24 hours without inverting the plates, creating optimal conditions for the growth of both the test organisms and any potential inhibition zones that would indicate antimicrobial activity.

3.5. **Extract preparation**

The selected actinobacteria, following an initial antimicrobial screening, were carefully inoculated into 700 mL of ISP1 medium, which was adjusted to a pH of 7.2 to promote optimal growth. This process was performed in a 500 mL conical flask, all procedures were carried out under sterile conditions to avoid contamination. Once inoculated, the flasks were placed on a shaker incubator set to a speed of 120 rpm, allowing for efficient aeration and mixing, and were kept at room temperature for a duration of one week. As fermentation progressed over the week, turbidity was developed in the medium, indicating active microbial growth and metabolic activity. Upon completion of the fermentation period, the culture was harvested and subjected to centrifugation to effectively separate the cells and any debris from the liquid culture. To extract the bioactive secondary metabolites, the resulting broth was mixed with an equal volume of solvent, utilizing a 1:1 ratio (v/v) of methanol. This mixture was allowed to sit for 24 hours, facilitating the extraction process. Following this period, the solvents were carefully filtered out using Whatman filter paper to ensure clarity of the extract. The excess solvent from the filtrate was then evaporated under reduced pressure using rotary evaporator, resulting in the concentration of the crude extract, ready for further analysis and potential applications.

3.6. **Secondary antimicrobial screening of Endophytic Actinobacteria**

The selected actinobacterial extracts were subjected to antimicrobial screening against 15 strains of clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) pathogens, as detailed in Table 3.2. These strains were collected from patients at Synod Hospital

in Durtlang, Aizawl, Mizoram. The agar well diffusion method was used for the antimicrobial testing. A concentration of 20 mg/mL of the crude extract was prepared for the experiment and 4% methanol solution was used as a negative control. Using sterile cork borers, wells measuring 6 mm in diameter were created on an agar plate. The test organism was swabbed onto the agar surface, and 100 µL of the 20 mg/mL crude extract was then added to each well (Boyanova, 2005). Vancomycin was used as a positive control and the plates were allowed to rest for a few minutes before incubation at 28°C for 24 hours, without inverting them. The diameters of the zone of inhibition were measured in millimeters.

Table 3.2: Test organisms for secondary antimicrobial screening

S.no	Pathogens	Organisms	Resistance
1.	038	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
2.	161	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
3.	365	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
4.	452	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
5.	622	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
6.	645	*Staphylococcus aureus*	Rifampicin, Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

7.	790	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
8.	809	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
9.	924	*Staphylococcus aureus*	Rifampicin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
10.	1098	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
11.	1246	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
12.	1266	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
13.	1344	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
14.	1379	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
15.	1458	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

3.7. **Morphological identification using Gram staining and Scanning Electron Microscope**

A clean glass slide was prepared with a smear of culture and carefully heated over a flame. The smear was subsequently coated with a light layer of crystal violet and left to sit for one minute before being gently rinsed with slow-running tap water. Following this, Gram's iodine solution was applied to the smear for 1 minute and then rinsed off with tap water. The smear was then decolorized with alcohol until the violet colour no longer flowed away. After rinsing with water again, a counterstain of safranin was applied for 2 minutes. Finally, the slide was washed, drained, air-dried, and examined under a microscope, 10× and 40× magnification and then under 100× with oil immersion. (Kumar et al., 2010). The spore chain structures of the isolates were analysed using a scanning electron microscope (SEM). The filamentous structures were inspected with a phase contrast microscope (Olympus). The identification of the organisms was carried out based on the 9th edition of Bergey's Manual of Determinative Bacteriology (Barka et al., 2016).

3.8. Characterization of potent strains using 16S rRNA gene sequencing

Genomic DNA was isolated and purified using a DNA extraction kit (Invitrogen) according to the protocol given by the manufacturer. Using universal bacterial primers 16S rRNA genes were amplified. 16SP1 and 16SP2 as forward and reverse primer (forward 16s rRNA primer 5'- GTGCCAGCAGCCGCGG- 3' and reverse 16s rRNA primer 5'-TACGGYTACCTTGTTACDACTT- 3') (Tailliez et al., 2006). The reactions and conditions of the PCR were performed in a Veriti thermal cycler (Applied Biosystem, Singapore) in a total volume 25µL and the PCR conditions are as follows: initial denaturation step at 94◦C for 5 min, followed by 35 cycles of denaturation at 94◦C for 1 min, annealing at 62◦C for 1 min and extension at 72◦C for 1.2 min with a final extension step at 72◦C for 5 min and sequencing was done commercially at Eurofins Pvt. Ltd. Bangalore, India. The sequences were analysed against reference strains of actinobacteria from the NCBI genomic database using the BLASTN search tool to assess similarity percentages. The strains exhibiting the highest similarity percentages were obtained from the NCBI database.

3.9. **Phylogenetic analysis of MMSK12 and MMSK47**

The sequences of the 16S rRNA gene were compared with the NCBI database using BLASTN and the most similar match sequence was selected. Type strains were obtained from Ez taxon database. The sequences were aligned with pair wise alignment and multiple alignment using the program Clustal W packaged in the MEGA 11 software (Tamura et al.,2021). From this data, a phylogenetic tree was constructed using the neighbor-joining tree method (Saitou and Nei, 1987). Bootstrap analysis was performed with MEGA 11 using Tamura-Nei (TN93) (Tamura and Nei, 1993).

3.10. Antioxidant assay using DPPH (1,1-Diphenyl-2-picryl-hydrazyl)

The DPPH assay was conducted following the methodology described by Villano et al. (2007). 2mg/mL concentration was prepared as a stock solution for each extract and then diluted to concentrations ranging from 5 to 1000 µg/mL. An aliquot of 50 µL from each dilution was transferred into a 96-well microtiter plate (Tarsons, Kolkata, India). A fresh solution of 0.1 mM DPPH in methanol was prepared, and 200 µl of the prepared solution was added to each well. After thorough mixing, the mixture was allowed to react and was then incubated in a dark room for 30 minutes. The absorbance of the mixture was measured at 517 nm using a Thermo Scan Go microplate reader (Thermo Scientific, USA). An extract without DPPH served as the blank, methanol was used as control and ascorbic acid was used as positive control. The IC_{50}, which denotes the concentration of antioxidant required to achieve a 50% reduction in DPPH, was calculated using a calibration curve based on linear regression. Results were expressed as the percentage reduction of DPPH absorption compared to the control. The percentage of DPPH reduction was calculated using the equation:

$$\% \text{ Reduction} = (\text{Abs of control} - \text{Abs of sample}) / \text{Abs of sample} * 100$$

Where Abs of sample is the absorbance of the test sample and Abs of control is the absorbance of the control.

3.11. ABTS radical scavenging assay

The ABTS+ radical scavenging capacity of the extract was measured using the 96-well microtiter plate method (Re et al., 1999). A stock solution of each extract at a concentration of 2 mg/mL was prepared and then diluted to concentrations ranging from 5 to 1000 µg/mL. 150 µL from each dilution was transferred to the 96-well microtiter plates. A solution of 7 mM ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) and 2.45 mM potassium persulfate (1:1) was prepared using distilled water. This solution was incubated for 12 to 16 hours and then diluted with methanol to achieve an absorbance reading of 0.7 at 734 nm, using a Thermo Scan Go microplate reader (Thermo Scientific, USA). To each well containing the extracts, 150 µL of the prepared ABTS solution was added and mixed thoroughly. The plates were then made to react in a dark room for 7 minutes, after which the absorbance was measured at 734 nm. An extract without ABTS served as the blank, and methanol served as control and ascorbic acid was used as positive control. The IC_{50} was calculated using a calibration curve based on linear regression. Results were expressed as the percentage radical scavenging of ABTS absorption compared to the control.

The percentage of and ABTS+ radical scavenging was calculated by using the equation:

% Scavenging= $(A_{control}-A_{sample})/ A_{sample} X100$

Where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

3.12. **Determination of Total Phenolic Content**

Total phenolic content was determined by using the Folin Ciocalteu assay (Singleton 1965). 2mg of extract was weight and 1mL of methanol was added and vortexed until the extract was dissolved. 1mL of FC reagent was taken in a conical flask and 10 mL of autoclaved H_2O was added. 30mL of extract was then mixed with 150µL of F.C reagent and then incubated for 5 minutes. Na2CO3 (75gm/L) was prepared for 30mL. Here 2.25gm of Na2CO3 was weight and added in 30mL of water. 120µL of Na2CO3 was added in the solution (extract + buffer) and incubated for 90-120 minutes. Absorbance was then taken at 725 and 760nm. TPC was calculated from the following equation:

TPC=C X V/M

Where,

TPC= Total phenolic content mg/g of extracts as GAE

C= concentration of gallic acid established from the calibration curve in mg/mL

V= volume of the extracts solution in mL

M= weight of the extracts in gram

3.13. **Determination of Total Flavonoid Content**

The aluminium chloride colorimetric assay method was used for quantification of the total flavonoid content of the methanolic extracts (Zhishen et al., 1999). Here 1M of $AlCl_3$ was first added in 150µL of Ethanolic $AlCl_3$ ($AlCl_3$=133.34mg) .150µL of plant extract was mixed with 150µL of $AlCl_3$. The solution was then incubated for 30 minutes and absorbance was taken at 420/430nm. In milligrams of quercetin equivalents per gram of dry material, flavonoid amounts were expressed. Triplicates of each determination were made. Using MS Excel software, correlation coefficients (R^2) were determined to assess the link between two variables. TFC was calculated from the following equation:

TFC=C X V/M

Where,

TFC= Total flavonoid content mg/g of extracts as QE

C= concentration of quercetin established from the calibration curve in mg/mL

V= volume of the extracts solution in mL

M= weight of the extracts in gram

3.14. **MIC determination using plate-based method**

The determination of the minimum inhibitory concentration (MIC) using the plate-based method was done using the agar well diffusion technique. In this process, methanolic extracts from the various actinobacterial strains were prepared with various concentrations of 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 mg/mL. The different concentrations were tested against 5 clinical MRSA pathogens. Each concentration of

the extract was then carefully placed into the wells within the agar plates, while methanol was used as a negative control to measure the effectiveness of the extracts. After the incubation period of 24 hours at a temperature of 28°C. The efficacy of the extracts was assessed by measuring the zone of inhibition, the clear halo zone surrounding the wells which indicates the antimicrobial activity.

3.15. **MIC determination using broth microdilution method**

The Minimum Inhibitory Concentration (MIC) was assessed using the broth microdilution technique, using the procedure given by Eloff in 1998. Methanolic extracts of the actinobacterial strains were prepared and diluted to different concentrations (1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 mg/mL) to evaluate their antimicrobial activity against 5 clinical MRSA pathogens in microtiter plates. The normal growth of the pathogen without the extract served as control for comparison. The plates were allowed to incubate at 28 °C for 24 hours, after which absorbance was measured at 600 nm using a UV-Vis spectrophotometer (MultiscanTM GO, Thermo Scientific, MA, USA). The effective concentration for 50% inhibition (EC_{50}) was calculated using GraphPad Prism.

3.16. **Compound separation using Thin Layer Chromatography (TLC)**

Fraction was done using TLC based on polarity (Shoun et al., 1998). In which four different types of solvent system were used namely:

solvent system 1: (Butanol-Acetic acid-Water, 30:20:70 v/v)

solvent system 2 (Chloroform-Methanol, 24:1 v/v)

solvent system 3 (Ethyl acetate-Methanol, 6:4 v/v)

solvent system 4 (Hexane-Ethyl acetate, 1:9 v/v)

TLC was carried out on commercially available aluminum TLC sheets (20 × 20 cm with 0.2 mm thickness, silica gel GF254, Merck, Darmstadt, Germany). The solvent fronts were marked and Rf values were measured using the formula,

Retention factor (Rf)= distance travelled by the solute/ distance travelled by the solvent.

3.17. **Antimicrobial validation of active compounds against MDR isolates**

The selected potent compounds underwent testing against multidrug-resistant clinical isolates utilizing the agar well diffusion assay, as described by Saadoun and Muhana in 2008. In this process, pathogenic microbes were carefully inoculated onto a nutrient agar plate. A thin-layer chromatography (TLC) plate, which contained the isolated compounds, was then gently positioned on top of the inoculated media. The assembled plates were incubated at a stable temperature of 28 ± 2°C for a duration of 24 hours, and the zone of inhibition was observed after 24 hours. To ensure reliable results, all experiments were done in triplicates.

3.18. **Gas Chromatography Mass Spectroscopy analysis**

For the gas chromatography-mass spectrometry (GC–MS) analysis, a Clarus 690 Perkin Elmer gas chromatograph was coupled with a Turbomass Gold 5.1 mass spectrometer. An Elite 1 capillary column, made of 100% dimethyl polysiloxane and measuring 123.5 m x 0.678 mm, was used to identify the volatile organic compounds (VOCs) in the methanolic extracts of *Streptomyces* sp. The instrument was set to 40ºC, then raised to 150ºC at 10ºC per minute for 5 minutes. It was subsequently increased to 250ºC at 40ºC per minute and held for 8 minutes. The injection port temperature was set at 250ºC, and the helium flow rate was maintained at 1.5 mL/min. An ionization voltage of 70 eV was used with a 10:1 split mode for sample injection. The mass spectral scanning range was set from m/z 500 to 800. The ion source and interface temperatures were maintained at 230ºC and 240ºC, respectively. The mass spectrometry (MS) data collection started at 3 minutes and ended at 31 minutes, with a solvent cut time of 3 minutes. The spectra of the volatile compounds detected through GC-MS were compared and matched with the NIST17 online library, version 2.339.

3.19. **High Performance Thin Layer Finger print profile**

The extract underwent further analysis utilizing the CAMAG HPTLC instrument, which was paired with the winCAT software for data management. To prepare for the analysis, a stock solution was created by dissolving 50 mg of the sample in distilled water to achieve a concentration of 50 mg/mL. For the chromatography process, TLC plates measuring 2 x 10 cm and coated with 0.2 mm silica gel from Merck, Germany,

were selected for the experiment. The sample was carefully spotted onto the plates using the Linomat 5 automated sample spotter from CAMAG, which employed a 100 µL Hamilton syringe for precise application. Three solvent systems were utilized as mobile phase designed specifically for profiling phenolics, glycosides, and anthracenes, ensuring that the separation was effective for each compound type. To facilitate the drying of the bands, nitrogen gas was applied to the plates during the process. The plates, once loaded with the extracts, were placed in a TLC twin trough developing chamber filled with the chosen mobile phase. After the development was complete, the plates were thoroughly dried and subsequently scanned using a TLC scanner with the winCAT software for analysis. Following the drying process, the plates were directly visualized under UV light at wavelengths of 254 nm and 366 nm, allowing for the detailed examination and documentation of the unique fingerprint profiles of the extracts. Key data points, including peak displays, peak densitograms, and peak tables, were recorded to ensure comprehensive analysis and interpretation of the results.

CHAPTER 4

RESULTS

RESULTS

4.1. Selection and collection of medicinal plants

A comprehensive literature review led to the selection of five traditional medicinal plants native to the state of Mizoram. These plants are *Tithonia diversifolia*, known for its vibrant yellow flowers and various health benefits; *Centella asiatica* (L.) Urb., well known for its healing properties and its extensive used in skin care; *Mikania micrantha*, a vigorous vine with notable medicinal applications; *Mirabilis jalapa* L., commonly referred to as the four o'clock flower, recognized for its therapeutic uses; and *Clerodendrum colebrookianum* Walp., a shrub valued in traditional healing practices. These species are illustrated in Figure 4.1, highlighting their significance in local herbal medicine.

Fig 4.1: Pictorial representation of the selected medicinal plants A) *Mirabilis jalapa* B) *Clerodendrum colebrookianum* Walp C) *Mikania micrantha* D) *Centella asiatica* (L.) E) *Tithonia diversifolia*

4.2. Isolation of Endophytic Actinobacteria

To obtain maximum diversity of actinobacteria five nutritional media were used, each selected for its potential to support actinobacterial growth. The media used are SCA (starch casein agar), AIA (actinomycetes isolation agar), tyrosine agar medium (ISP7), glycerol asparagine agar base (ISP5), and King's medium. From this, 54 distinct actinobacterial isolates were successfully isolated from five traditionally recognized medicinal plants found in the region of Mizoram. Among the various media tested, the highest levels of actinobacterial growth were observed in ISP7, which shows its efficacy for cultivating these microorganisms. The growth levels were subsequently followed by SCA, AIA, ISP5, and King's medium in terms of their ability to sustain the isolates (Fig4.3). Each individual isolate was arranged using a systematic numbering system, labeled as MMSK1, MMSK2, MMSK3, and so forth, all the way up to MMSK54. The specific distribution of these isolates, linked to their respective plant sources, is represented in Fig. 4.2. The plant *Mirabilis jalapa* L. yielded the greatest number of isolates (n=18), This was closely followed by *Centella asiatica* (L.) Urb, which contributed 15 isolates and *Mikania micrantha* with 11 isolates, *Tithonia diversifolia* with 6 isolates, and *Clerodendrum colebrookianum* Walp with 4 isolates, highlighting the variability in endophytic actinobacterial diversity among these valuable medicinal plants.

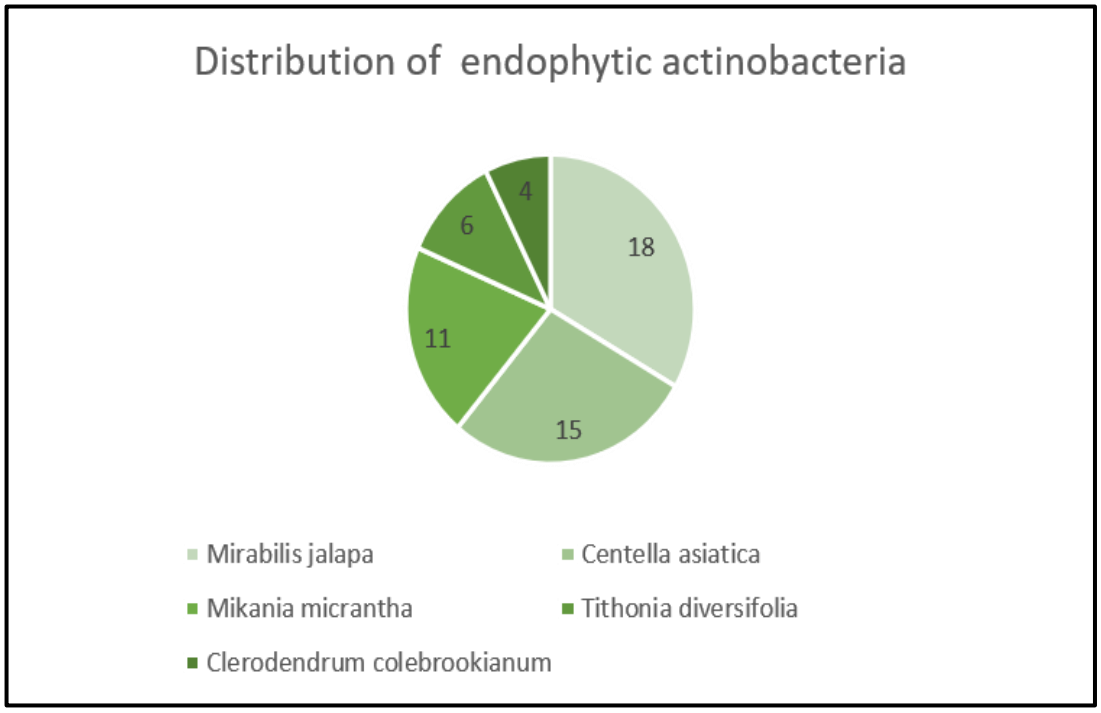


Fig 4.2: Distribution of endophytic actinobacteria among the five selected traditional plants of Mizoram

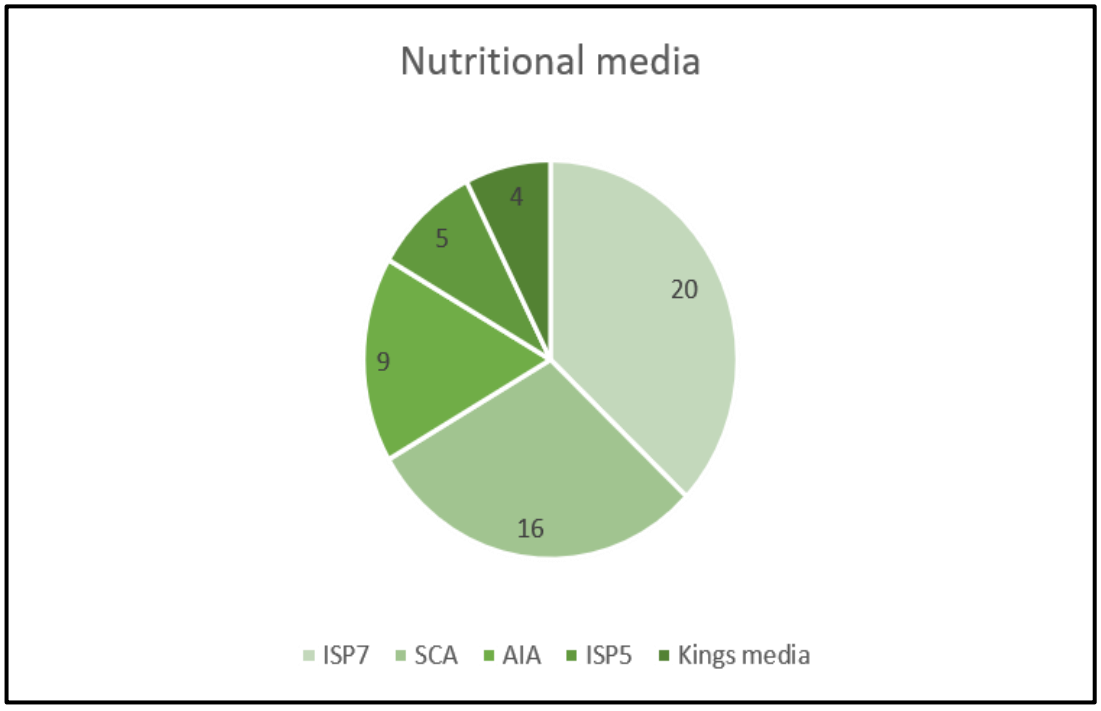


Fig 4.3: Yield of nutritional media for the growth of endophytic actinobacteria

4.3. Primary antimicrobial screening of Endophytic Actinobacteria

A total of 54 endophytic actinobacteria isolates underwent initial antimicrobial screening against 33 clinical multidrug-resistant (MDR) pathogens. For this primary screening, the supernatant from three-five days old broth cultures grown in ISP1 media was used. To conduct the assay, 100 µL of the collected supernatant was carefully allotted into pre-formed wells within agar plates inoculated with the pathogens. The initial results revealed that 32 of the 54 isolates exhibited significant inhibitory effects against three or more of the tested pathogens (Fig 4.4). From these,15 isolates were selected based on the inhibition potential for further investigation. The detailed results observed for all isolates are documented in Table 4.1.

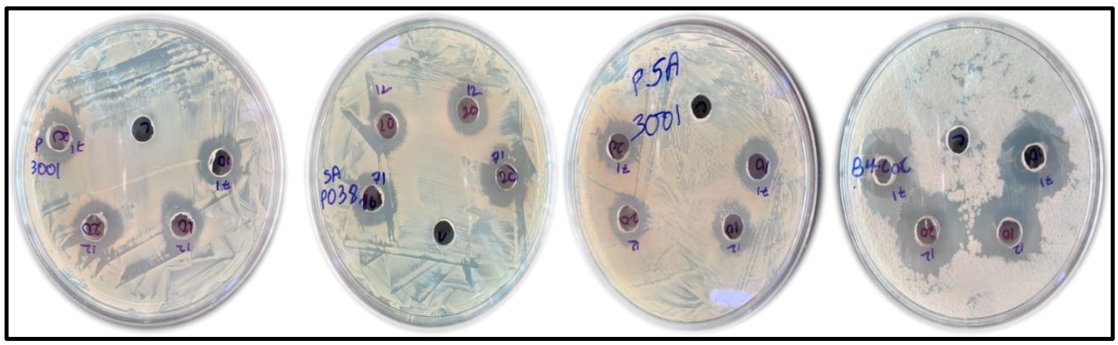


Fig 4.4: Zone of inhibition in primary antimicrobial screening

Table 4.1: Results of primary antimicrobial screening of 32 actinobacterial isolates against 33 pathogens

Pathogens	**MMSK1**	**MMSK2**	**MMSK4**	**MMSK7**	**MMSK8**	**MMSK9**	**MMSK11**	**MMSK12**	**MMSK13**	**MMSK14**	**MMSK15**
17	+	-	+	-	-	-	-	-	-	-	-
31	-	-	-	-	-	+	-	-	-	-	-
038	-	+	-	+	-	+	-	-	+	+	+
161	-	-	-	+	-	-	-	+	-	-	+
363	-	-	-	-	-	-	-	-	-	-	-
365	-	-	-	-	-	-	+	+	+	+	+
452	-	-	-	-	-	-	-	+	-	+	-

536	+	-	-	-	-	-	-	-	-	-	-
622	-	-	-	+	-	-	+	+	-	+	+
645	-	-	-	+	-	-	+	+	+	-	-
721	-	-	-	-	-	-	-	-	-	-	-
790	-	-	-	+	-	+	-	+	-	+	+
809	-	-	-	-	-	-	+	+	-	-	-
880	-	-	-	-	-	-	-	-	-	-	-
924	-	-	-	-	-	-	+	+	-	-	+
995	+	-	-	-	-	-	-	-	-	-	-
1097	-	+	-	-	-	-	-	-	-	-	-
1098	-	-	-	-	+	-	-	+	+	+	-
1219	-	-	-	-	-	-	-	-	-	-	-
1246	-	-	+	+	-	+	-	+	+	-	-
1258	-	-	-	-	-	-	-	-	-	-	-
1266	+	+	-	+	-	-	+	-	+	+	-
1344	-	-	-	-	+	-	-	-	-	+	+
1379	-	-	-	+	-	+	+	-	-	-	+
1458	-	-	-	+	-	-	+	+	-	-	-
1468	-	-	-	-	-	-	-	-	-	-	-
1536	-	-	-	-	-	-	-	-	-	-	-
2822	-	-	-	-	-	-	-	-	-	-	-
2982	-	-	-	-	-	-	-	-	-	-	-
2997	-	-	-	-	-	-	-	-	-	-	-
3001	-	-	-	-	+	-	-	-	-	-	-
3054	-	-	+	-	+	-	-	-	-	-	-
3069	-	-	-	-	-	-	-	-	-	-	-

Pathogens	MMSK17	MMSK19	MMSK21	MMSK23	MMSK24	MMSK28	MMSK31	MMSK34	MMSK35	MMSK36	MMSK37
17	-	-	-	-	-	-	-	-	-	+	-
31	-	-	-	-	-	-	-	-	-	-	-
038	-	-	-	-	+	-	-	+	-	-	-
161	+	+	-	-	-	-	-	+	+	+	-
363	-	-	-	-	-	+	-	-	-	-	-
365	+	-	-	+	+	-	+	-	+	-	+
452	+	-	+	+	-	-	-	+	-	-	-
536	-	-	-	-	-	-	-	-	-	-	-
622	-	-	+	-	+	-	-	+	+	-	-
645	+	+	-	+	-	-	-	+	-	-	-
721	-	-	-	-	-	+	-	-	-	+	-
790	-	-	-	+	-	-	-	+	-	-	-
809	-	-	-	+	+	-	+	-	+	-	-
880	-	-	-	-	-	+	-	-	-	-	-
924	+	-	-	-	-	+	-	-	-	-	-
995	-	-	-	-	-	-	-	-	-	-	-
1097	-	-	-	-	-	-	-	-	-	-	-
1098	+	-	-	-	-	-	-	+	+	-	-
1219	-	-	-	-	-	-	+	-	-	+	-
1246	-	-	-	+	+	-	-	+	+	-	-
1258	-	-	-	-	-	-	+	-	-	-	-
1266	+	-	-	+	-	-	-	-	-	-	-

1344	+	-	-	-	+	-	-	-	+	-	+
1379	-	-	-	+	-	-	-	+	-	-	-
1458	+	+	-	-	+	+	-	-	+	-	-
1468	-	-	+	-	-	-	-	-	-	-	-
1536	-	-	+	-	-	-	-	-	-	-	-
2822	-	+	-	-	-	-	-	-	-	-	-
2982	-	-	-	-	-	-	-	-	-	+	-
2997	-	+	+	-	-	-	-	-	-	-	-
3001	-	-	-	-	-	-	-	-	-	+	-
3054	-	-	-	-	-	-	-	-	-	-	+
3069	-	-	-	-	-	-	-	-	-	-	+

Pathogens	**MMSK38**	**MMSK40**	**MMSK41**	**MMSK44**	**MMSK45**	**MMSK47**	**MMSK49**	**MMSK51**	**MMSK52**	**MMSK54**
17	-	-	+	-	+	-	-	-	-	+
31	-	-	-	-	-	-	-	+	-	-
038	-	-	-	+	-	+	-	-	+	-
161	-	+	-	+	-	+	-	-	-	-
363	-	-	-	-	-	-	+	-	-	-

365	-	+	-	+	-	+	+	-	+	-
452	-	+	-	-	-	+	-	-	+	-
536	-	-	+	-	-	-	-	-	-	-
622	-	+	-	-	-	+	-	-	+	-
645	-	-	-	+	-	-	-	-	+	+
721	+	-	+	-	-	-	-	-	-	-
790	-	+	-	-	-	+	-	-	+	+
809	-	+	-	+	-	-	+	-	-	-
880	-	-	-	-	-	-	-	-	-	-
924	-	+	-	-	+	+	-	-	-	-
995	-	-	-	-	-	-	-	+	-	-
1097	-	-	+	-	-	-	-	-	-	-
1098	-	-	-	+	-	+	-	+	+	-
1219	-	-	-	-	-	-	-	-	-	+
1246	-	-	-	-	-	+	-	-	-	+
1258	+	-	-	-	-	-	-	-	-	-
1266	-	+	-	+	-	-	-	-	+	+
1344	-	-	-	+	-	-	-	-	-	-
1379	-	-	-	+	-	+	-	-	-	-
1458	-	+	-	-	-	+	-	-	-	-
1468	-	-	+	-	-	-	-	-	-	-
1536	+	-	-	-	-	-	-	-	-	-
2822	+	-	-	-	-	-	+	-	-	-
2982	-	-	-	-	-	-	-	-	-	-
2997	-	-	-	-	+	-	-	-	-	-
3001	-	-	-	-	-	-	-	-	-	-
3054	-	-	-	-	+	-	-	-	-	-
3069	-	-	+	-	-	-	-	-	-	-

4.4. Extract preparation

During the primary antimicrobial screening, a total of 32 positive isolates were identified, showcasing promising inhibition potential. From these, 15 of the most effective isolates were carefully selected for the preparation of extracts intended for secondary screening. The extracts were prepared using methanol as the solvent, resulting in an approximate yield of 1 to 2 grams of extract from each of the chosen isolates. This process ensured that each extract retained the unique properties of its corresponding isolate, setting the stage for further evaluation of their antimicrobial efficacy.

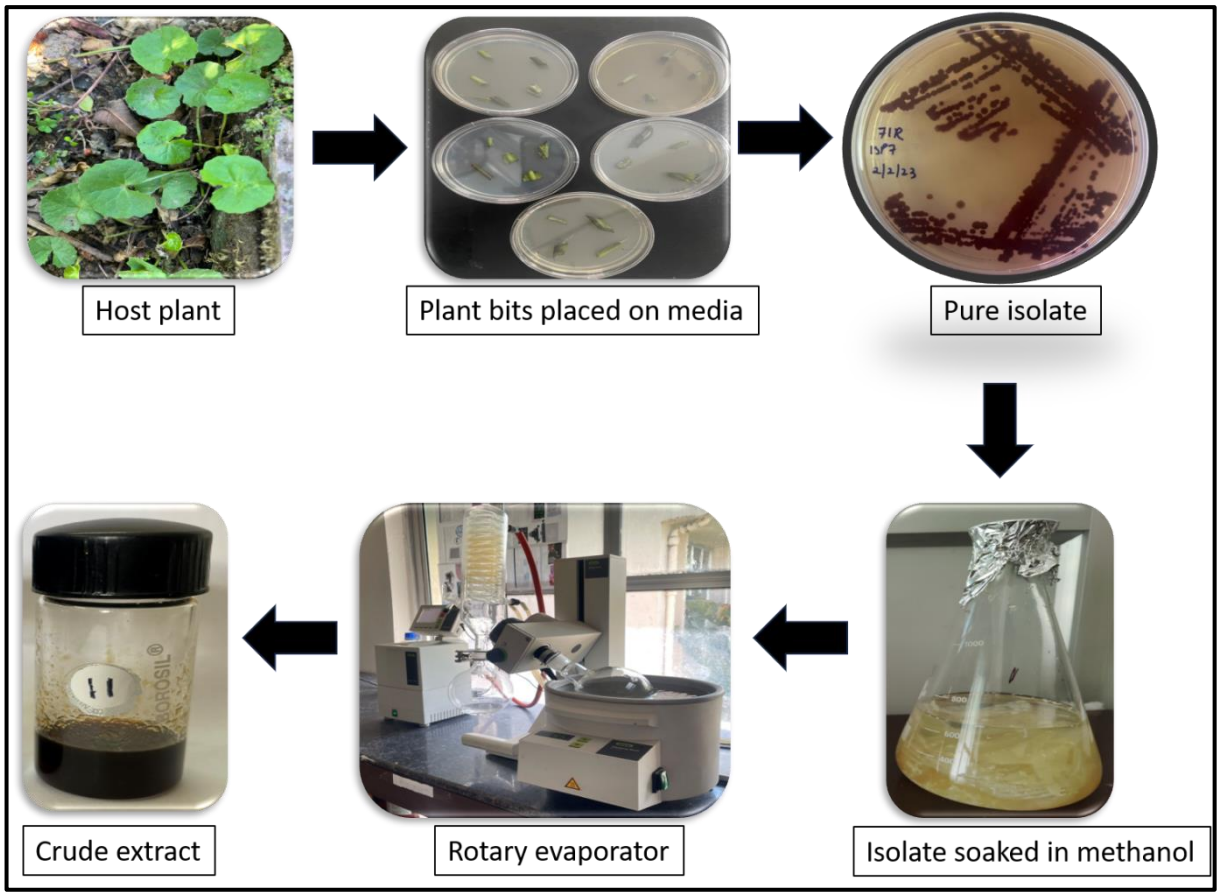


Fig 4.5: Process of making crude extract

4.5. Secondary antimicrobial screening of Endophytic Actinobacteria

Based on the primary antimicrobial screening, 15 isolates were selected for secondary antimicrobial screening using 20mg/mL of the crude extract against 15 MRSA

(Methicillin resistant *Staphylococcus aureus*) clinical pathogens. All of the isolates have antimicrobial properties against at least to 6 of the tested pathogens. Among these isolates, MMSK12 and MMSK47 have shown the most potential inhibition, exhibiting the ability to inhibit 11 of the tested pathogens. The zone of inhibition for these isolates varied significantly, ranging from 4mm to 16mm in diameter. MMSK12 and MMSK47 were selected for further analysis.

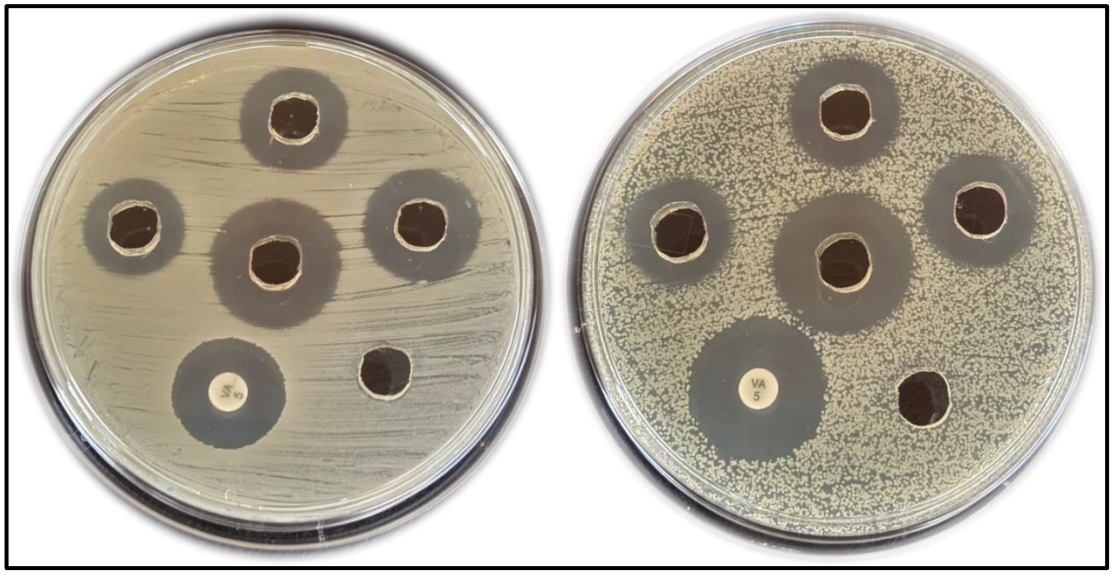


Fig 4.6: Secondary antimicrobial screening using agar well diffusion method with vancomycin as positive control

Table 4.2: Zone of inhibition in mm exhibited in secondary antimicrobial screening

Pathogens	**Zone of inhibition in mm**				
	MMSK7	**MMSK11**	**MMSK12**	**MMSK13**	**MMSK14**
038	6	-	-	6	6
161	6	-	4	-	-
365	-	4	14	6	6
452	-	-	4	-	8
622	8	6	4	-	8
645	6	6	6	8	-
790	6	-	10	-	4
809	-	8	4	-	-

924	-	4	10	-	-
1098	-	-	14	4	4
1246	4	-	12	4	-
1266	8	8	-	6	4
1344	-	-	-	-	6
1379	4	2	-	-	-
1458	10	8	6	-	-

Pathogens	**Zone of inhibition in mm**				
	MMSK15	**MMSK17**	**MMSK23**	**MMSK24**	**MMSK34**
038	4	-	-	2	4
161	4	4	-	-	4
365	4	6	10	6	-
452	-	4	4	-	6
622	6	-	-	6	6
645	-	4	6	-	8
790	4	-	8	-	8
809	-	-	4	8	-
924	6	8	-	-	-
1098	-	8	-	-	6
1246	-	-	4	8	8
1266	-	6	6	-	-
1344	6	6	-	10	-
1379	4	-	4	-	4
1458	-	6	-	10	-

Pathogens	Zone of inhibition in mm				
	MMSK35	**MMSK40**	**MMSK44**	**MMSK47**	**MMSK52**
038	-	-	8	6	6
161	4	4	6	10	-
365	8	4	4	16	6
452	-	6	-	10	6
622	4	6	-	8	4
645	-	-	2	-	2
790	-	4	-	12	6
809	8	6	6	-	-
924	-	6	-	16	-
1098	10	-	8	14	8
1246	8	-	-	14	-
1266	-	8	8	-	6
1344	8	-	6	-	-
1379	-	-	8	8	-
1458	8	4	-	6	-

4.6. Morphological identification using gram staining and Scanning Electron Microscope

Both MMSK12 and MMSK47 have been identified as gram-positive bacteria, exhibiting a purple coloration when examined under the microscope. Observations made using a scanning electron microscope have revealed the intricate details of their spore surfaces, which presented a smooth texture characterized by branched, filamentous structures. Furthermore, these microorganisms demonstrated the ability to form microspore chains, resulting in the development of distinct and complex colonies.

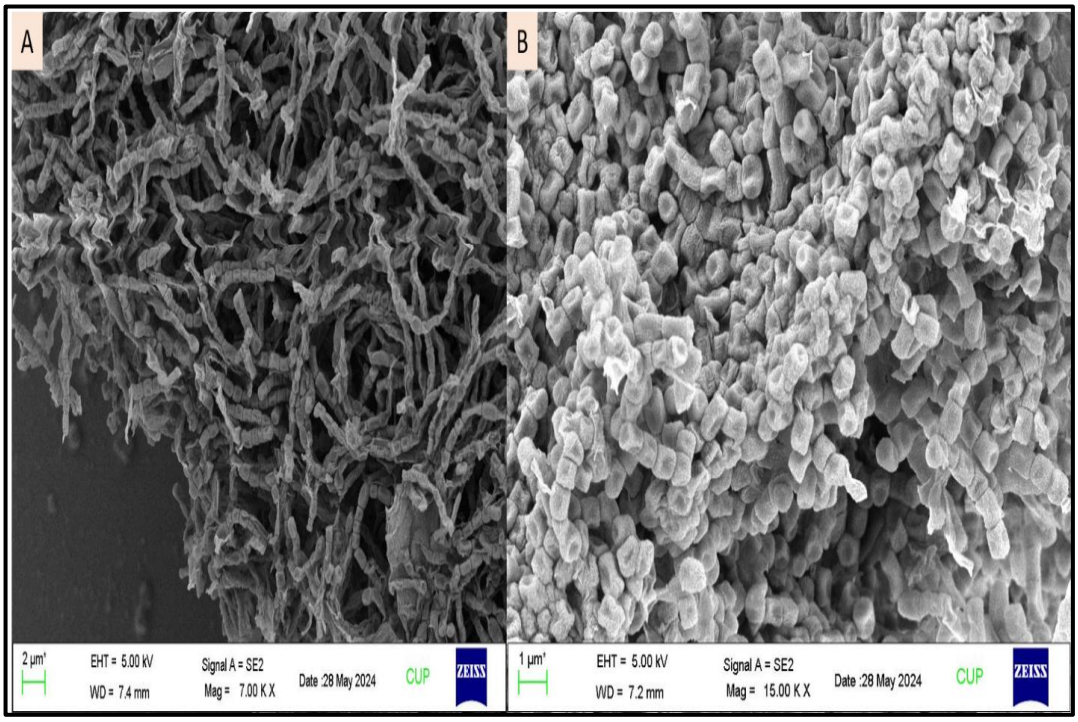


Fig 4.7: A) MMSK12 B) MMSK47 viewed under scanning electron microscope

4.7. Characterization of potent strains using 16S rRNA gene sequencing

The two most promising actinobacterial isolates, identified as MMSK12 and MMSK47, were selected for further analysis based on the secondary antimicrobial screening. MMSK12 and MMSK47 were analysed for molecular identification using 16S rRNA gene sequencing, a reliable method for classifying bacteria based on genetic material. The detailed analysis indicated that isolate MMSK47 is part of the genus Streptomyces, showing 99.3% genetic similarity to *Streptomyces* sp. NSC30, as evidenced by the sequencing results (MG833381). Similarly, isolate MMSK12 was also classified within the Streptomyces genus, demonstrating the same high percentage of 99.3% similarity with *Streptomyces* sp. QJSt7 (KX950906). The sequences obtained from these molecular identifications have been deposited in the NCBI GenBank database, with accession numbers OR896092 for MMSK47 and OR896079 for MMSK12.

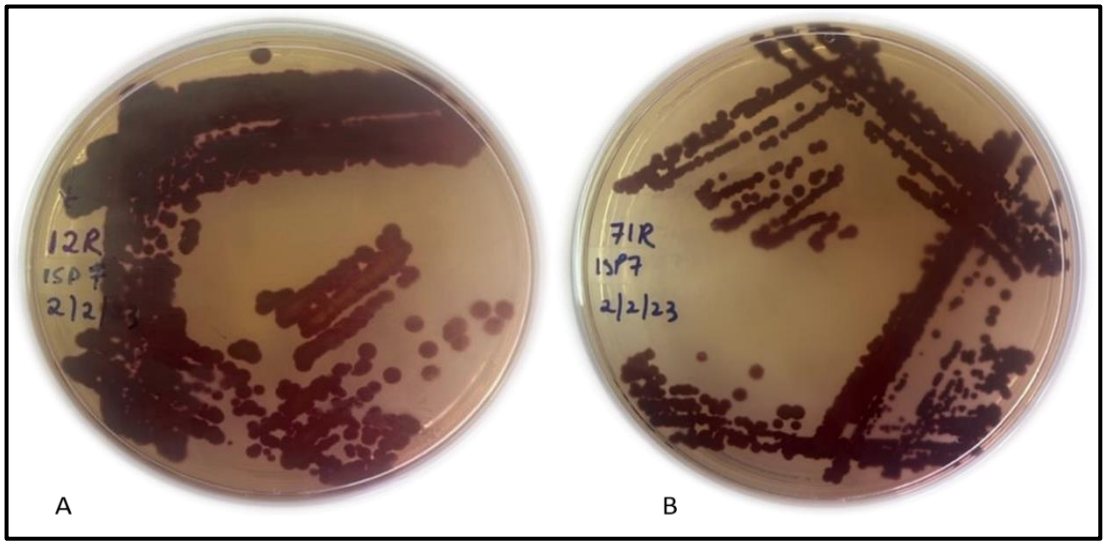


Fig 4.8**:** Pure isolates of the two selected potential strains A) MMSK12 and B) MMSK47

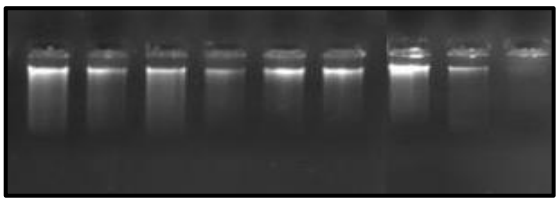


Fig 4.9: DNA of MMSK12 and MMSK47 viewed under Gel Doc in triplicates

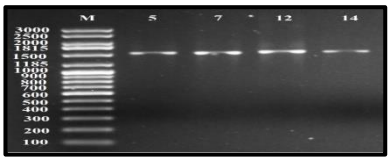


Fig 4.10: Amplified PCR product

4.8. Phylogenetic analysis of MMSK12 and MMSK47

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. The rate variation

among sites was modeled with a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

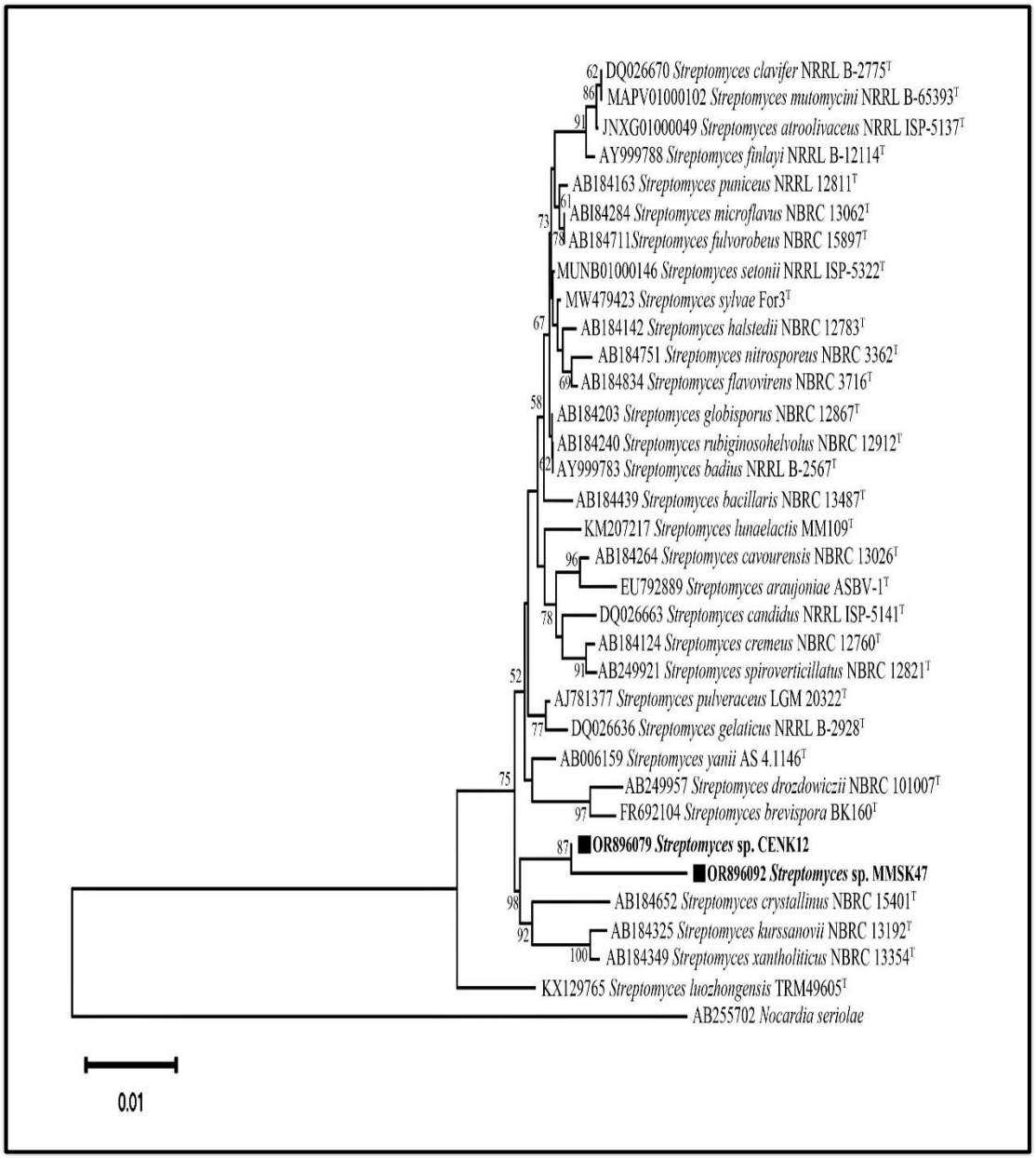


Fig 4.11: Phylogenetic tree for MMSK12 and MMSK47 constructed using Neighbor Joining method using Tamura Nei Model

4.9. DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay

The antioxidant potential of MMSK12 and MMSK47 was evaluated by examining their ability to scavenge DPPH free radicals, a common method for assessing radical

scavenging activity. This process involves measuring the degree of decolorization of the DPPH solution, which occurs as the compounds trap unpaired electrons from the radicals. Interestingly, the results indicated that the DPPH scavenging activity of both MMSK12 and MMSK47 was not as pronounced when compared to the values obtained from the ABTS assay, suggesting that their effectiveness in neutralizing free radicals might vary depending on the testing method used.

Table 4.3: IC_{50} value for DPPH assay

S.No	Samples	IC_{50} (µg/mL)
1.	MMSK12	668.3±0.92
2.	MMSK47	863.7±0.85
3.	Ascorbic acid	33.24±0.52

4.10. ABTS radical scavenging assay

The ABTS radical scavenging assay serves as a significant method for investigating the antioxidant properties of various crude extracts, and this study focuses specifically on the evaluation of MMSK12 and MMSK47 extracts. In this assay the antioxidant activity was measured through the calculation of the IC_{50} value, which represents the concentration of the extract needed to neutralize or eliminate 50% of the free radicals present in the ABTS solution. A lower IC_{50} value is indicative of a higher antioxidant capacity, signifying that a smaller amount of the extract is required to achieve effective free radical scavenging. The IC_{50} value for the MMSK12 extract was determined to be 82.78 µg/mL, while the MMSK47 extract exhibited an IC50 value of 94.14 µg/mL. Both extracts fall into the category of representing medium antioxidant activity, suggesting their significant potential in combating oxidative stress. Furthermore, these findings show the possible pathways for enhancing their antioxidant effectiveness.

Table 4.4: IC_{50} values for ABTS assay

S.No	Samples	IC_{50} (µg/mL)
1.	MMSK12	82.78±0.87
2.	MMSK47	94.14±0.80
3.	Ascorbic acid	14.65±0.92

4.11. Determination of Total Phenolic Content

The Phenolic Content of the MMSK12 and MMSK47 extracts was quantified using Gallic acid as the reference standard. To ensure accuracy, carefully prepared samples were subjected to analysis in relation to a pre-established standard curve utilizing UV-Vis Spectroscopy. The results of TPC analysis showed that the MMSK12 extract contains a concentration of 68.6±0.51 mg of Gallic Acid Equivalent (GAE) per gram, while the MMSK47 extract has a slightly lower but still significant level of 60.4±0.77 mg of GAE per gram. These findings showed the remarkable phenolic composition present in both extracts, suggesting their potential for various applications.

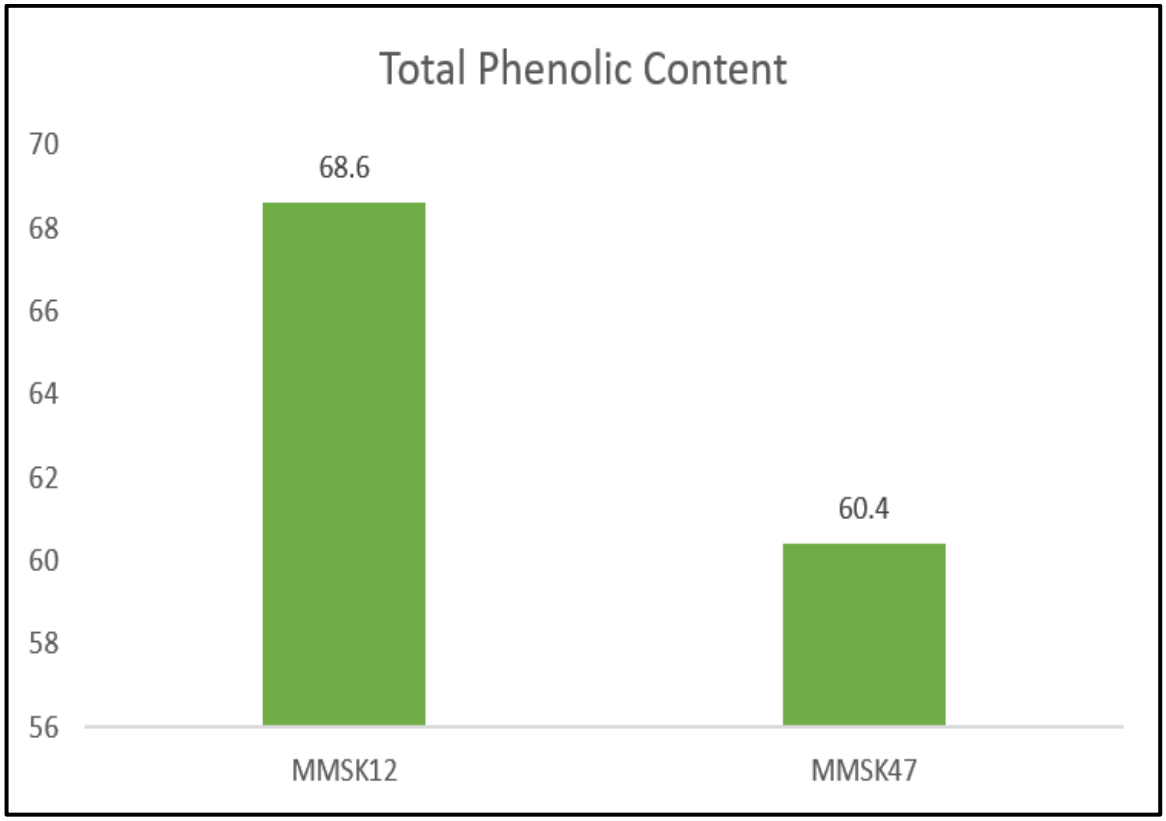


Fig 4.12: Chart for Total Phenolic Content of MMSK12 and MMSK47

Table 4.5: Total Phenolic Content of the best two isolates

S.No	Samples	Total phenolic content (mg GAE/g of sample extracts ±SD)
1.	MMSK12	68.6±0.27
2.	MMSK47	60.4±0.77

4.12. Determination of Total Flavonoid Content

A Quercetin standard curve, previously prepared and securely stored for future applications in UV-vis spectroscopy, was utilized to evaluate the flavonoid content present in the methanol extracts of the MMSK12 and MMSK47 samples. This analytical procedure yielded precise measurements of flavonoid concentrations, recorded at 10.92±0.64 mg of Quercetin (QE) per gram of the extract for MMSK12 and 12.89±0.73 mg of Quercetin (QE) per gram for MMSK47. These findings showed the substantial presence of flavonoid compounds in both the MMSK12 and MMSK47 extracts.

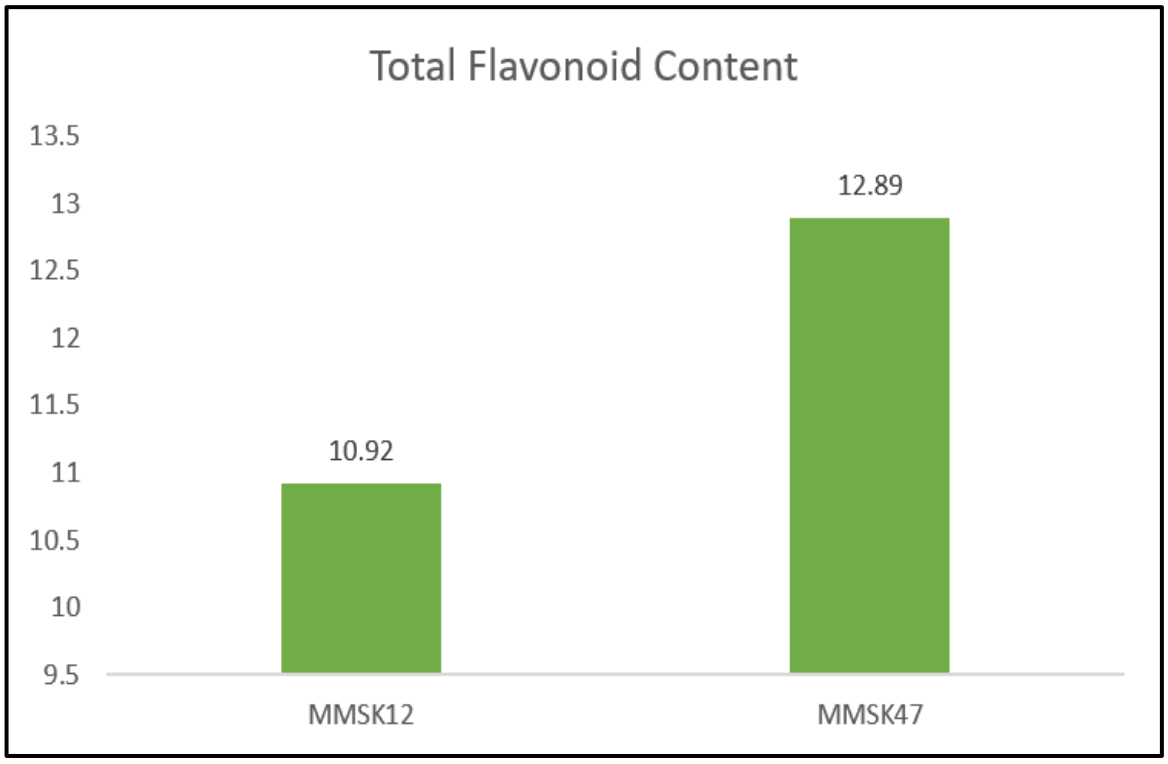


Fig 4.13: Chart for Total Flavonoid Content of MMSK12 and MMSK47

Table 4.6: Flavonoid Content of the best two isolates

S.No	Samples	Total flavonoid content (mg QE/g of sample extracts±SD)
1.	MMSK12	10.92±0.20
2.	MMSK47	12.89±0.73

4.13. MIC determination using plate-based method

The minimum inhibitory concentration (MIC) of the methanolic crude extract from MMSK12 and MMSK47 was evaluated against five clinical multidrug-resistant *Staphylococcus aureus* (MRSA) pathogens (365, 790, 924, 1098, and 1246), with the MIC determination concentrations ranging from 1 mg/mL to 18 mg/mL. The concentration of 20 mg/mL was omitted as it was utilized for secondary antimicrobial testing. The MIC zone of inhibition for MMSK12 was recorded at 1mg/mL for 365 pathogen, 2 mg/mL against two pathogens (924 and 1246), 4mg/mL for 1098 pathogen while it was observed at 12 mg/mL for pathogen 790 (Fig 4.15). In contrast, the MIC zone of inhibition for MMSK47 was found at 1 mg/mL for pathogens 365, 924, 1098, and 1246, and for pathogen 790, it was noted at 2 mg/mL (Fig 4.16). The recorded measurements of the zone of inhibition for each concentration against the five clinical MRSA pathogens are presented in Table 4.7

Table 4.7: Zone of inhibition for plate-based MIC determination for MMSK12

S.No	**Concentrations**	**Zone of inhibition in mm**				
		790 (A)	**365(B)**	**924(C)**	**1098(D)**	**1246(E)**
1.	1mg/mL	-	4±0.08	-	-	-
2.	2 mg/mL	-	7±0.08	3±0.04	-	4±0.03
3.	4 mg/mL	-	9±0.04	7±0.05	5±0.05	6±0.03
4.	6 mg/mL	-	10±0.05	7±0.04	8±0.06	7±0.04
5.	8 mg/mL	-	11±0.12	8±0.04	8±0.04	8±0.05
6.	10 mg/mL	-	11±0.10	8±0.08	8±0.04	9±0.03
7.	12 mg/mL	6±0.05	12±0.09	8±0.14	10±0.06	9±0.07
8.	14 mg/mL	7±0.10	12±0.08	9±0.12	10±0.10	9±0.05
9.	16 mg/mL	8±0.04	13±0.05	9±0.08	10±0.16	10±0.12
10.	18 mg/mL	9±0.05	13±0.05	9±0.08	11±0.06	10±0.08

Table 4.8: Zone of inhibition for plate-based MIC determination for MMSK47

S.No	Concentrations	Zone of inhibition in mm				
		790 (A)	365(B)	924(C)	1098(D)	1246(E)
1.	1mg/mL	-	4±0.03	2±0.10	2±0.09	2±0.05
2.	2 mg/mL	2±0.10	6±0.02	4±0.04	2±0.06	4±0.05
3.	4 mg/mL	4±0.05	8±0.03	6±0.06	3±0.10	6±0.07
4.	6 mg/mL	6±0.02	10±0.03	6±0.05	4±0.04	6±0.09
5.	8 mg/mL	6±0.07	12±0.10	8±0.03	4±0.08	8±0.04
6.	10 mg/mL	6±0.10	12±0.10	10±0.08	8±0.11	8±0.05
7.	12 mg/mL	8±0.02	14±0.06	10±0.12	10±0.03	10±0.17
8.	14 mg/mL	10±0.06	16±0.07	12±0.02	10±0.07	12±0.04
9.	16 mg/mL	12±0.10	18±0.05	12±0.10	12±0.09	14±0.10
10.	18 mg/mL	12±0.05	20±0.03	14±0.11	14±0.14	16±0.12

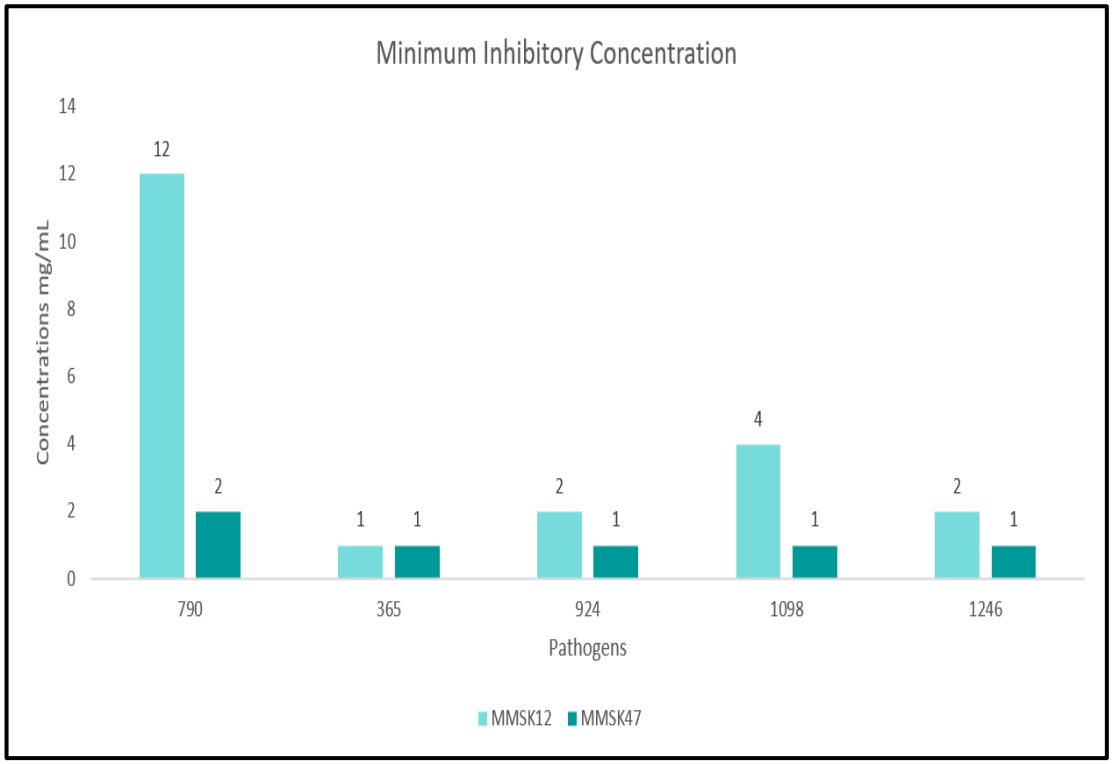


Fig 4.14 Chart for MIC values for MSSK12 and MMSK47 using plate-based method

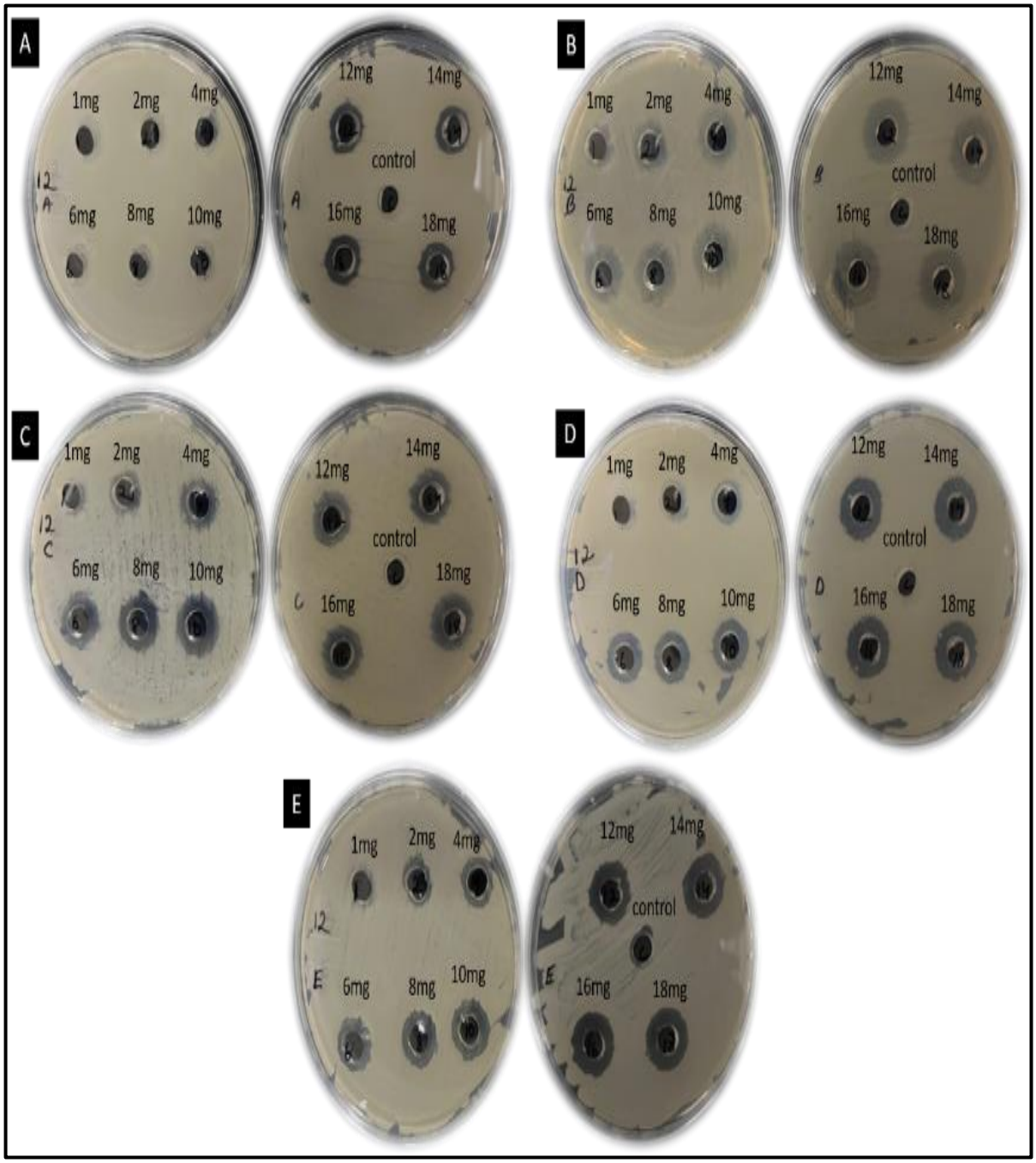


Fig 4.15**:** Zone of inhibition by different concentrations against five clinical MRSA pathogens for MIC determination of crude extracts of MMSK12

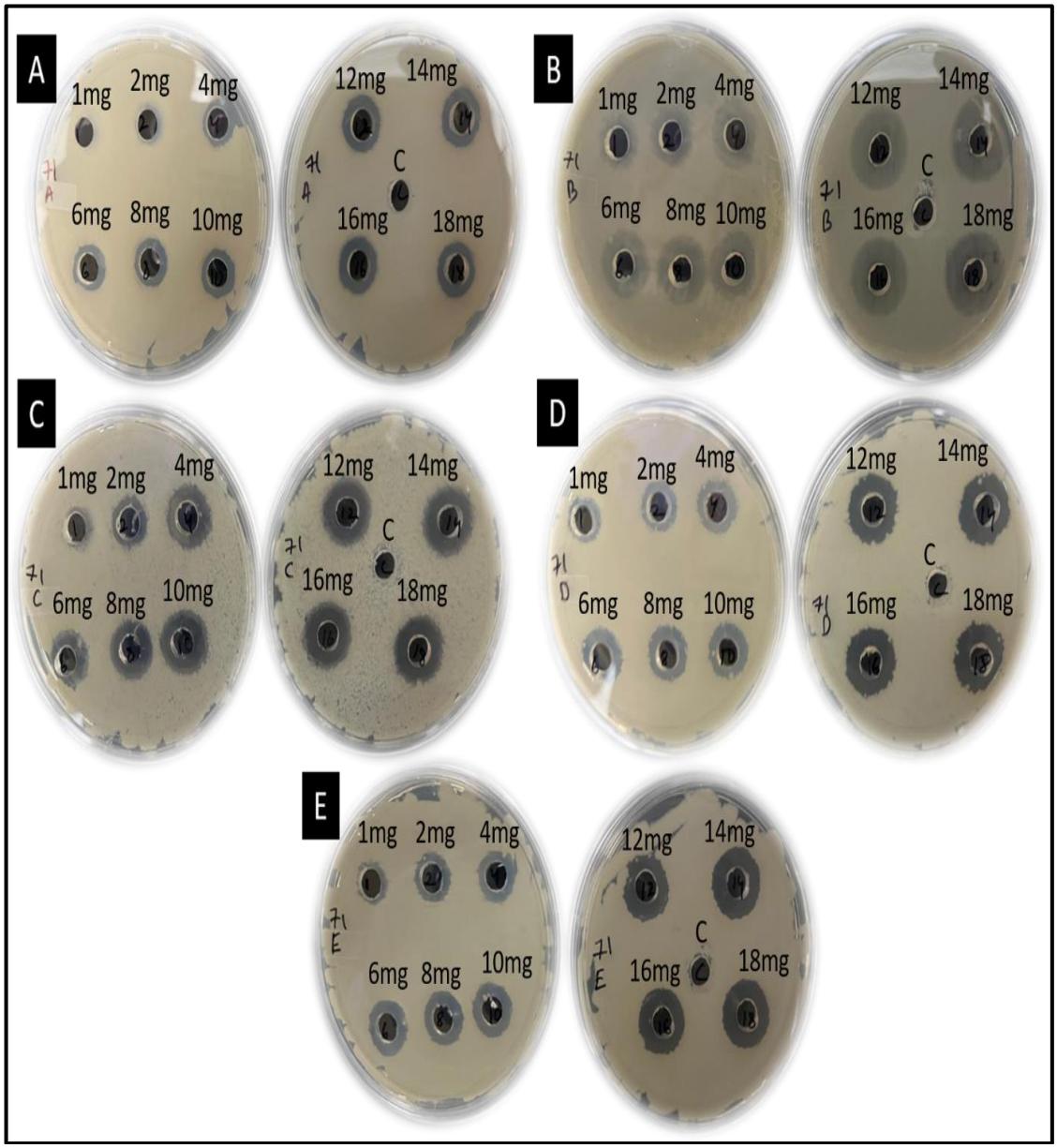


Fig 4.16**:** Zone of inhibition by different concentrations against five clinical MRSA pathogens for MIC determination of crude extracts of MMSK47

4.14. MIC determination using broth microdilution method

In the broth microdilution method employed for this study, a well-defined series of concentrations was utilized, which ranges from 1 mg/mL to 18 mg/mL. Both isolates, designated as MMSK12 and MMSK47, exhibited significant minimum inhibitory concentration (MIC) activity against each of the pathogens that were put to the test.

For the isolate MMSK12, the most remarkable antimicrobial activity was observed against pathogens 924, 1098, and 1246, with EC_{50} value of 2 mg/mL. This was followed by pathogen 365, which displayed a slightly reduced activity (EC_{50}=5 mg/mL), and pathogen 790, which had an EC_{50} of 6 mg/mL, indicating a diminishing efficacy in comparison to the other pathogens.

On the other hand, the isolate MMSK47 demonstrated even greater potency, particularly evident in its remarkable activity against pathogens 365, 924, 1098, and 1246, all of which had an EC_{50} value of just 1 mg/mL indicating a high level of effectiveness. Pathogen 790, while also showing sensitivity, had a higher EC_{50} value of 2 mg/mL, reflecting a slightly lower susceptibility compared to the other tested pathogens.

Table 4.9: MIC broth dilution EC_{50} value of MMSK12 and MMSK47

S.No	Samples	790(A)	365 (B)	924 (C)	1098 (D)	1246 (E)
1.	MMSK12	6 mg/mL	2 mg/mL	2 mg/mL	5 mg/mL	2 mg/mL
2.	MMSK47	2 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL

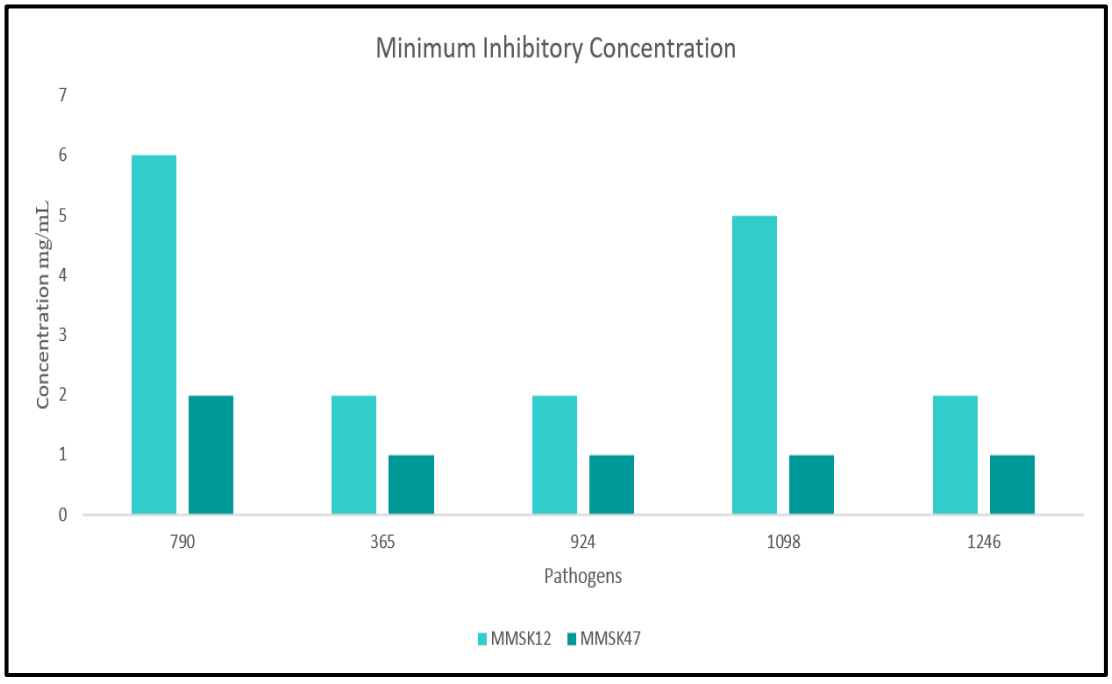


Fig 4.17**:** Chart for the Minimum Inhibitory Concentration for MMSK12 and MMSK47 using broth microdilution method

4.15. Fractionation of compounds using Thin Layer Chromatography (TLC)

Among the four solvent systems tested, one particular solvent system i.e. solvent system 1 (SS1) which is Butanol-Acetic acid-Water, 30:20:70 v/v was found to have the ability to achieve separation. As a result, it was selected for further separation experiments. The determined R_f value for MMSK12 was 0.54, indicating its migration distance in the chromatographic process, while MMSK47 exhibited an R_f value of 0.59, showcasing a slightly greater movement through the same solvent system. This variation in R_f values highlights the distinct characteristics of each compound in their interaction with the solvent.

4.16. Antimicrobial validation of active compounds against MDR isolates

The antimicrobial validation of active compounds against MRSA pathogens was done using a plate-based method. The results observed after 24 hours have shown a clear zone on the TLC plates where the separated compounds are present. This validates the antimicrobial activity of the different separated compounds.

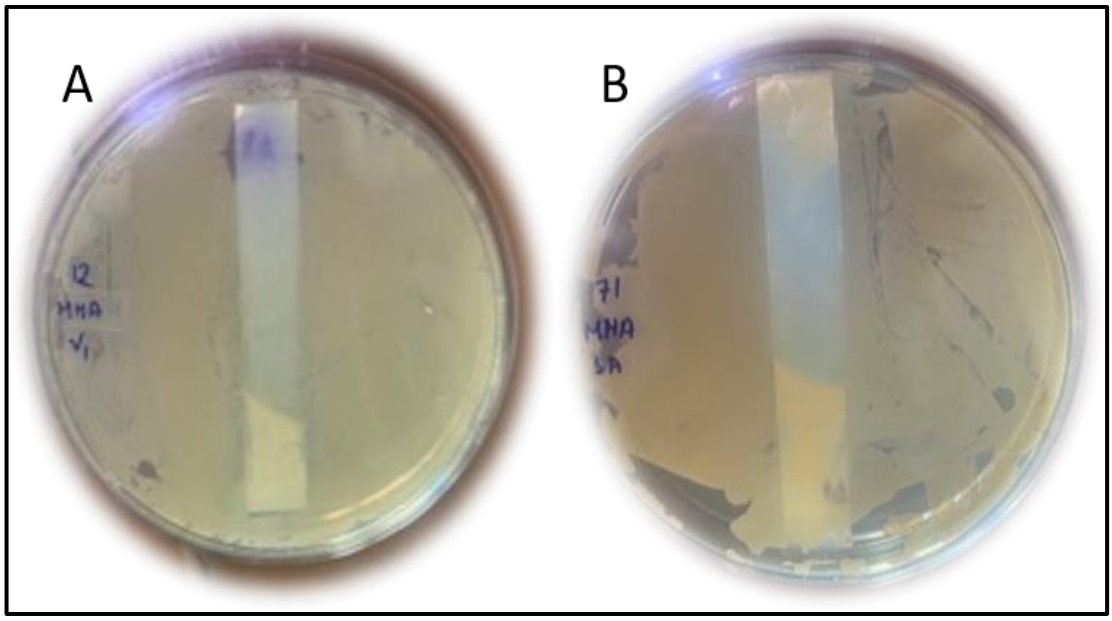


Fig 4.18: The clear zone formation on the TLC plates where the different compounds are present.

4.17. Gas Chromatography Mass Spectroscopy analysis

The methanol extract of *Streptomyces* sp. MMSK12 revealed a diverse number of 20 volatile organic compounds, each displaying a wide range of area percentages that varies from a mere 0.324% to 25.57% (Fig 4.19). Among these compounds, 1-Nenylcycloheptane emerged as the most abundant, dominating the composition of the extract with its significant presence which was followed by decanoic acid, which also contributed substantially to the overall profile of the extract.

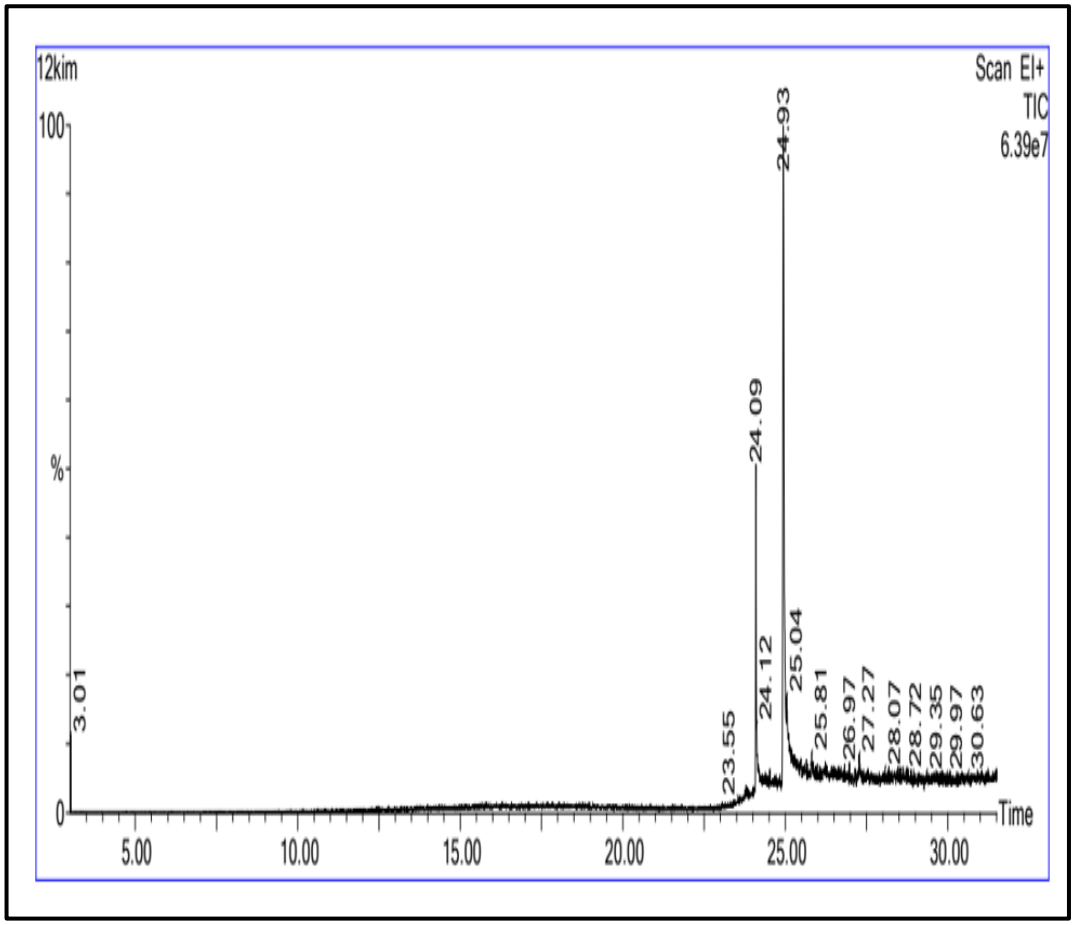


Fig 4.19**:** The GC-MS chromatogram of MMSK12 methanolic extract

Table 4.10: List of compounds detected in MMSK12 using GCMS

Sl no	RT	Area %	Name	Activities	Reference
1	24.092	7.979	N-Decanoic acid	Antimicrobial, anti tumor	Shen et al., 2021; Yang et al., 2023
2	24.522	0.395	1,4-Naphthoquinone, 6ethyl-2,3,5,7, tetrahydroxy	Antimicrobial, Antioxidant	Ravichandiran et al., 2019; Wu et al., 2020
3	24.932	25.572	1, Nenylcycloheptane	-	
4	25.042	6.319	Cycloheptylmine, N--Heptafluorobytyryl-	--	
5	25.197	1.063	2-hexanoic acid, (E)	Antimicrobial, Antioxidant	Huang et al., 2011; Aranega-Bou et al., 2014
6	25.262	0.444	Trans-1-4-D1-Tert-Butyl-cyclohexane	-	
7	25.293	0.524	1,2,3,4,5- cyclopentane pentol	Anti tumor, Anti viral	Tikhomirov et al., 2024; Smee et al., 2001
8	25.333	0.464	Anthracine, 9,10-dihydro-9-10-Bis(trimethylsilyl)	Antioxidant, Antimicrobial	Gonzalez et al., 2021; Kim et al., 2009
9	25.383	0.519	Acetohydrazine,2-denrylthio-N2-(Thiene-3yloxy0Benzylidenol	Antimicrobial, antioxidant	Popiolek,2021; Boulebd et al., 2022
10	25.483	0.585	4-Tart-Octylphenol, THS-derivatives	-	
11	25.813	0.514	1,3-Dioxolane, 2-pentadecyl	Antibacterial and antifungal,	Küçük et al., 2011; Raskildina et

				Anti oxidant	al., 2024
12	26.193	0.417	Adamantane-2,6-dione, Bis(ethyleneketal)	-	
13	26.968	0.480	3-Pentanal, 3 methyl	-	
14	27.268	1.451	Hexamethylene diacrylate	-	
15	27.538	0.387	4-Bromo-2-(Trifuomethoxy)Thiophenol,S-trimethylsilyl-	-	
16	28.359	0.325	1,4-cyclohexadiene,1,3,6-Tris (trimethylsilyl)	-	
17	29.294	0.324	1,3-Dioxolane, 2-pentadecyl	Antibacterial and antifungal, Anti oxidant	Küçük et al., 2011; Raskildina et al., 2024
18	29.349	0.593	1,3-Bis-T-Butylperoxy-phthalan	-	
19	29.414	0.438	3-Octanal,3,7-Dimethyl	Antioxidant, Antimicrobial	Matos et al., 2019; Wood and Szewczak, 2007
20	29.549	0.396	3,4-Methylenedioxyphenylacetic acid; TMS Derivative	-	

The methanolic crude extract of *Streptomyces* sp. MMSK47 was analysed and revealed a total of 19 volatile organic compounds (VOCs) as depicted in Fig 4.20. These compounds were detected within a retention time range of 15 to 29 minutes, indicating a diverse metabolic profile. The detailed area percentages of these VOCs, as outlined in Table 4.11, which demonstrate varying levels of abundance. Notably, 1H-indole-3-acetic acid 5-chloro-2-methyl-1-(trimethylsilyl) silane, exhibited the highest area percentage at 10.111%. In contrast, Perylene was found to be the least abundant VOC, with an area percentage of only 0.835%. This variation in VOC composition highlights the potential biochemical diversity of *Streptomyces* sp. MMSK47 and its possible applications in various fields, including biochemistry and pharmaceuticals.

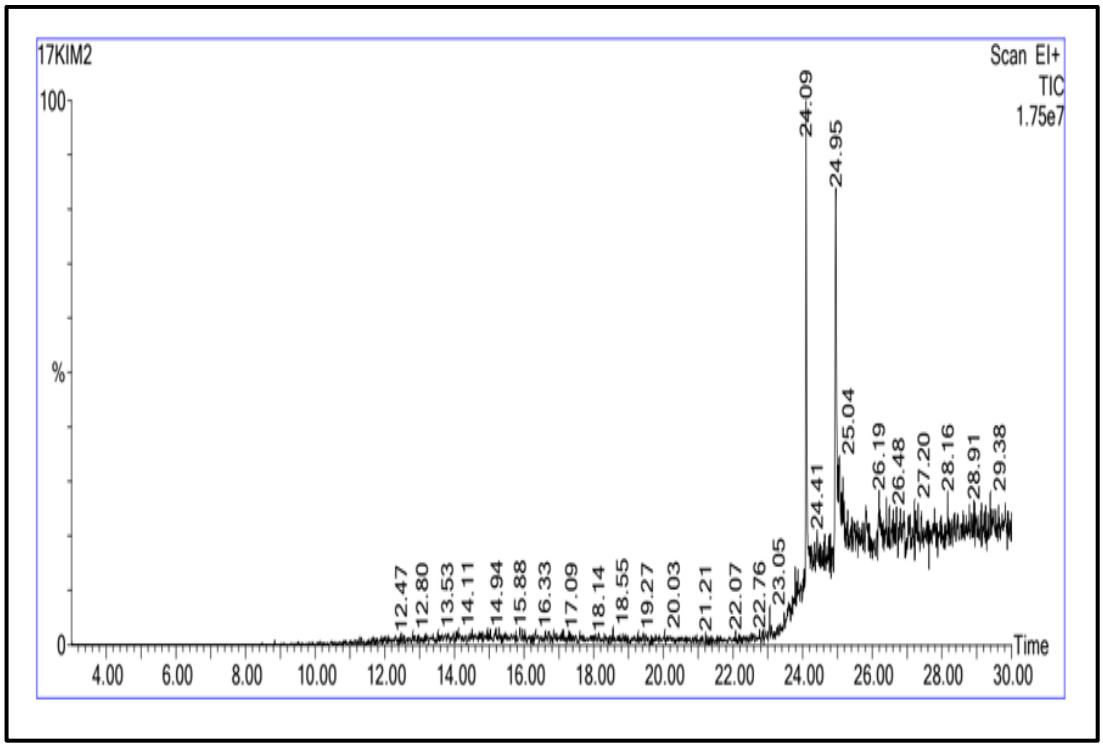


Fig 4.20: GC-MS chromatogram of MMSK47 methanolic extract

Table 4.11: List of compounds detected in MMSK47 using GCMS

S.no	RT	Area%	Name	Activity	Reference
1	15.88	0.835	Perylene	Antimicrobial, Antioxidant	Yılmaz et al., 2023; Rostaminejad et al., 2023
2	23.052	7.478	Spiro (2,4) Hept-S-ene, S-trimethyl-1-trimethysilyl-	-	
3	23.592	10.111	1H-indole-3-acetic acid, 5-chloro-2-methyl-1-(trimethylsilyl) silane	Antimicrobial, Antioxidant	Chitra et al., 2017
4	23.78	2.640	N- decanoic acid	Antimicrobial, anti tumor	Shen et al., 2021; Yang et al., 2023
5	24.09	0.845	Anthracene, 9,10, dihydro-9,10-Bis (trimethylsilyl)	Antimicrobial	Kim et al., 2009
6	24.412	1.636	Acethlydrazine, N2-{-4-(Thitan-3-yloxy) Benzylideno}	Antimicrobial	Popiołek et al., 2017
7	24.792	1.457	1-Nonylcycloheptane	-	
8	24.953	2.303	1,3-Dioxolane, 2-pentadecyl-	Antibacterial and antifungal, Anti oxidant	Küçük et al., 2011; Raskildina et al., 2024
9	25.053	0.989	1,4-Naphthoquinone, 6-ethyl-2,3,5,7-tetrahydroxy-	Antimicrobial, antioxidant	Ravichandiran et al., 2019; Wu et al., 2020

10	25.293	1.138	Octanal, 7-methoxy-3-7-dimethyl-	Antifungal, antioxidant, antimicrobial	Li et al., 2021; Matos et al., 2019; Wood and Szewczak, 2007
11	25.413	1.612	Dinitrophenyl-L-isoleucylglycine	-	
12	25.813	0.898	3-pethanol-3, methyl	-	
13	25.913	0.882	Anthracene, 9,10, dihydro-9,10-Bis (trimethylsilyl)	Antimicrobial	Kim et al., 2009
14	26.193	0.925	Tetrasiloxane, decamethyl		
15	26.403	1.525	2-phenyl-1-Benzimidazolyl) acetic acid	-	
16	26.50	1.684	Anthracene, 9,10, dihydro-9,10-Bis (trimethylsilyl)	Antimicrobial	Kim et al., 2009
17	26.803	0.921	(2-Phenyl-1-Benzeimidazolyl) acetic acid	-	
18	27.143	1.107	1,3-Dioxolane, 2-pentadecyl-	Antibacterial and antifungal, Anti oxidant	Küçük et al., 2011; Raskildina et al., 2024
19	29.814	1.393	Anthracene, 9,10, dihydro-9,10-Bis (trimethylsilyl)	Antimicrobial	Kim et al., 2009

4.18. High Performance Thin Layer Finger print profile

Three solvent system was used for mobile phase namely ethyl acetate: methanol: water (20:3:2), cyclohexane: ethyl acetate: formic acid (4:6:1) and toluene: ethyl acetate: methanol: acetic acid (3:5:1:0.5) to detect glycosides (SS1), phenols (SS2) and anthracene (SS3) respectively at a volume of 8 µl of methanolic extract performed at UV 254 nm and 366 nm in visualizer and visible light and single brown spots were detected (Fig.2). The images were captured and scanned in CAMAG TLC Scanner using VisionCATS 3.2 SP2 software. The peak display, peak densitogram, and peak table were recorded and analysed and Rf values were recorded, and the occurrence of a positive result was noted at wavelengths of 254 nm and 366 nm. For MMSK47, 5 peaks were recorded in SS1 and SS3 whereas, 7 peaks were recorded in SS2 at 254nm. The peaks with Rf value and area (%) for MMSK47 are shown in Table 4.12, 4.13, and 4.14. The peak chromatogram is given in Fig: 4.22, 4.23 and 4.24. For MMSK12, 8 peaks were recorded in SS1 and SS2 and 5 peaks were recorded for SS3. The peaks with Rf value and area (%) for MMSK12 are shown in Table 4.15, 4.16 and 4.17 for SS1, SS2 and SS3 respectively and the peak chromatogram is given in Fig: 4.25, 4.26 and 4.27 for SS1, SS2 and SS3 respectively.

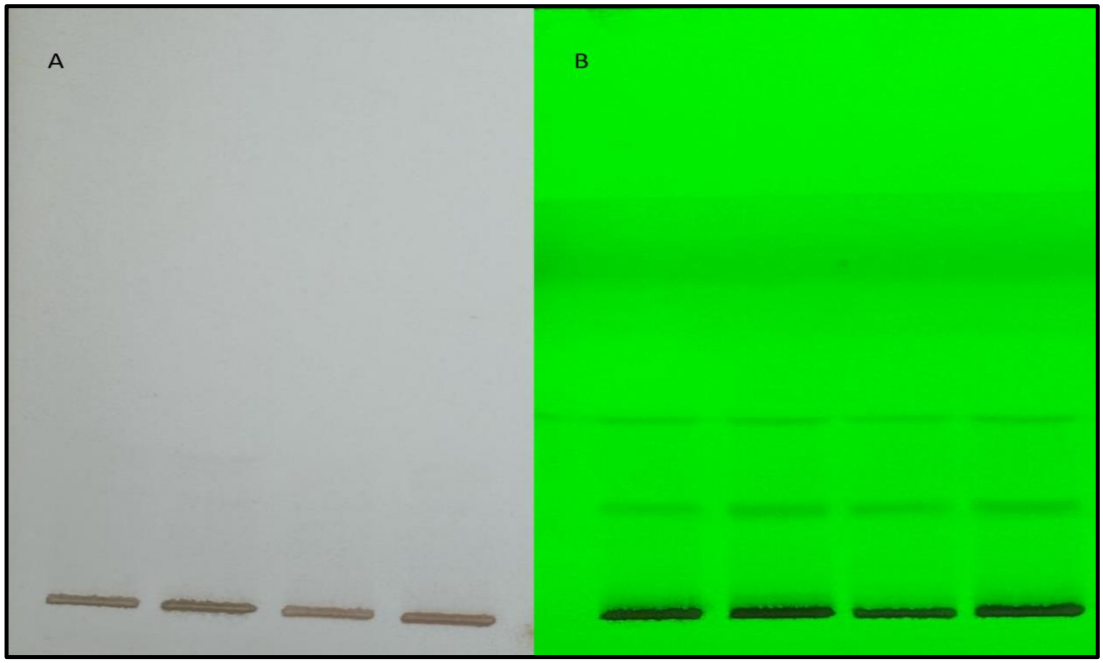


Fig 4.21: Visible light and single brown spots detected, captured and scanned in CAMAG TLC Scanner using VisionCATS 3.2 SP2 software

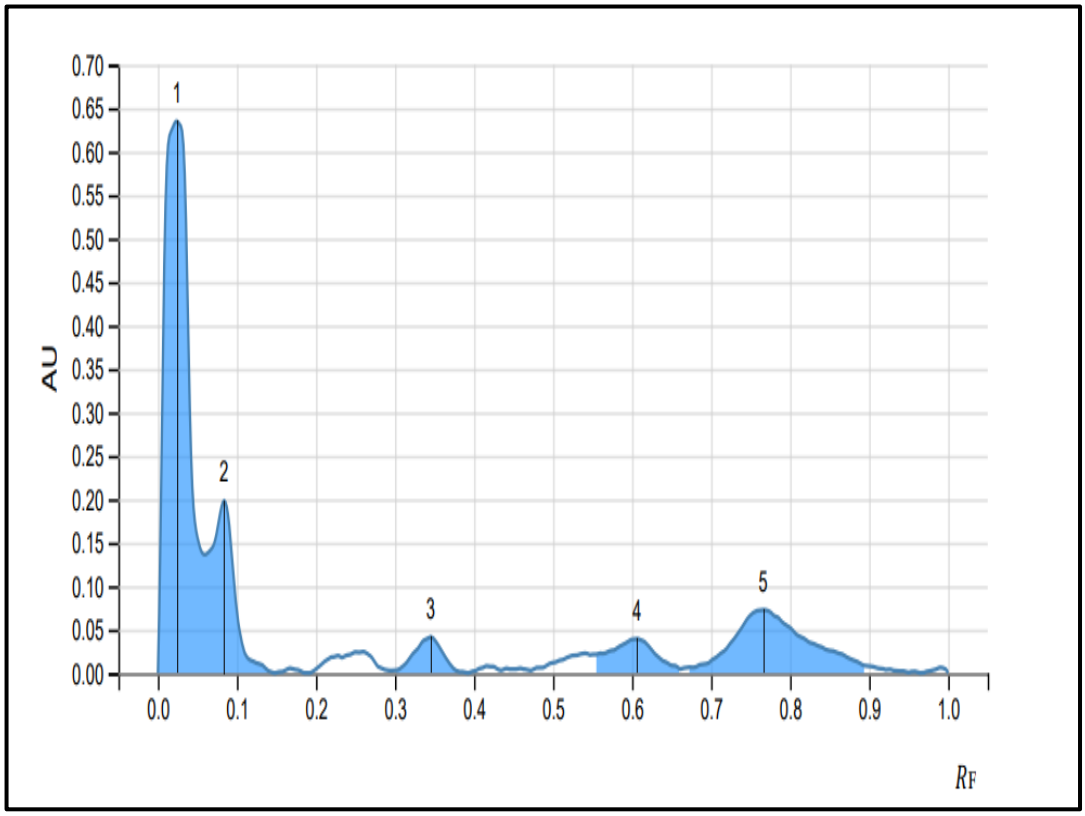


Fig 4.22: HPTLC chromatogram of 8 µl MMSK47 extract in SS1 performed at UV 254.

Table 4.12: R_F value and area percentage in HPTLC of SS1 of MMSK47

Peak	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.024	0.6345	64.35	0.060	0.1363	0.02380	55.03
2	0.060	0.1363	0.084	0.1979	20.08	0.147	0.0000	0.00700	16.18
3	0.295	0.0028	0.345	0.0415	4.21	0.384	0.0013	0.00173	4.00
4	0.555	0.0215	0.606	0.0394	3.99	0.661	0.0046	0.00265	6.14
5	0.669	0.0062	0.766	0.0727	7.37	0.895	0.0084	0.0084	18.65

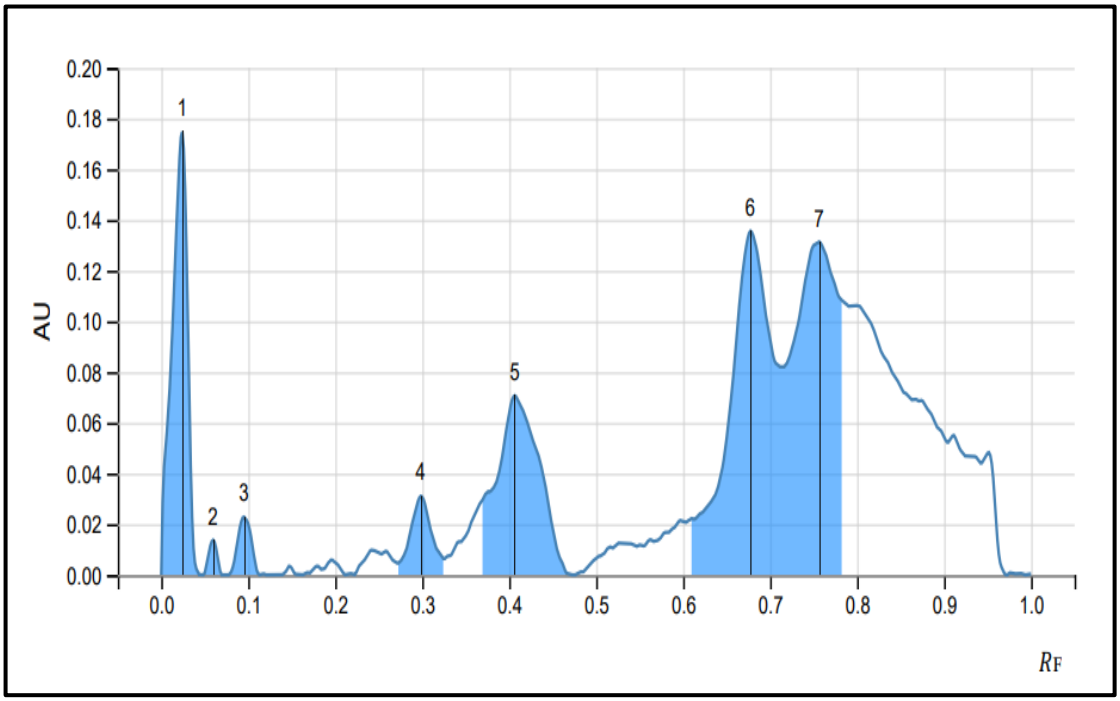


Fig 4.23: HPTLC chromatogram of MMSK47 SS2 at 254nm

Table 4.13: R_F value and area percentage in HPTLC of SS2 of MMSK47

Peak	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.024	0.1748	30.14	0.045	0.0000	0.00361	14.21
2	0.048	0.0000	0.060	0.0137	2.36	0.069	0.0000	0.00015	0.60
3	0.077	0.0000	0.095	0.0228	3.93	0.113	0.0000	0.00041	1.61
4	0.273	0.0046	0.298	0.0310	5.35	0.324	0.0065	0.00087	3.44
5	0.369	0.0295	0.406	0.0710	12.19	0.469	0.0003	0.00400	15.73
6	0.605	0.0210	0.677	0.1356	23.38	0.713	0.0819	0.00782	30.73
7	0.713	0.0819	0.756	0.1313	22.65	0.790	0.1060	0.00857	33.69

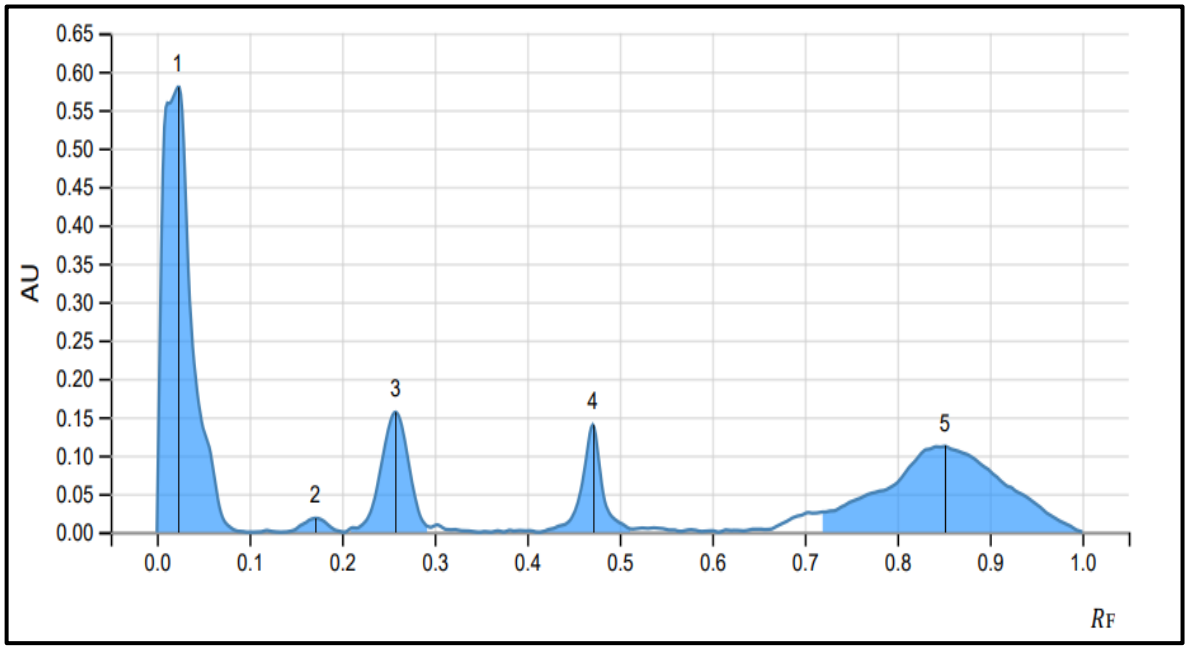


Fig 4.24: HPTLC chromatogram of MMSK47 SS3 at 254nm

Table 4.14: R_F value and area percentage in HPTLC of SS3 of MMSK47

Peak	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.023	0.5796	57.65	0.097	0.0000	0.02143	44.65
2	0.134	0.0000	0.171	0.0180	1.79	0.203	0.0000	0.00054	1.12
3	0.206	0.0022	0.258	0.1563	15.54	0.295	0.0063	0.00548	11.42
4	0.413	0.0000	0.471	0.1397	13.90	0.513	0.0040	0.00353	7.35
5	0.715	0.0252	0.852	0.1118	11.12	1.000	0.0002	0.01701	35.45

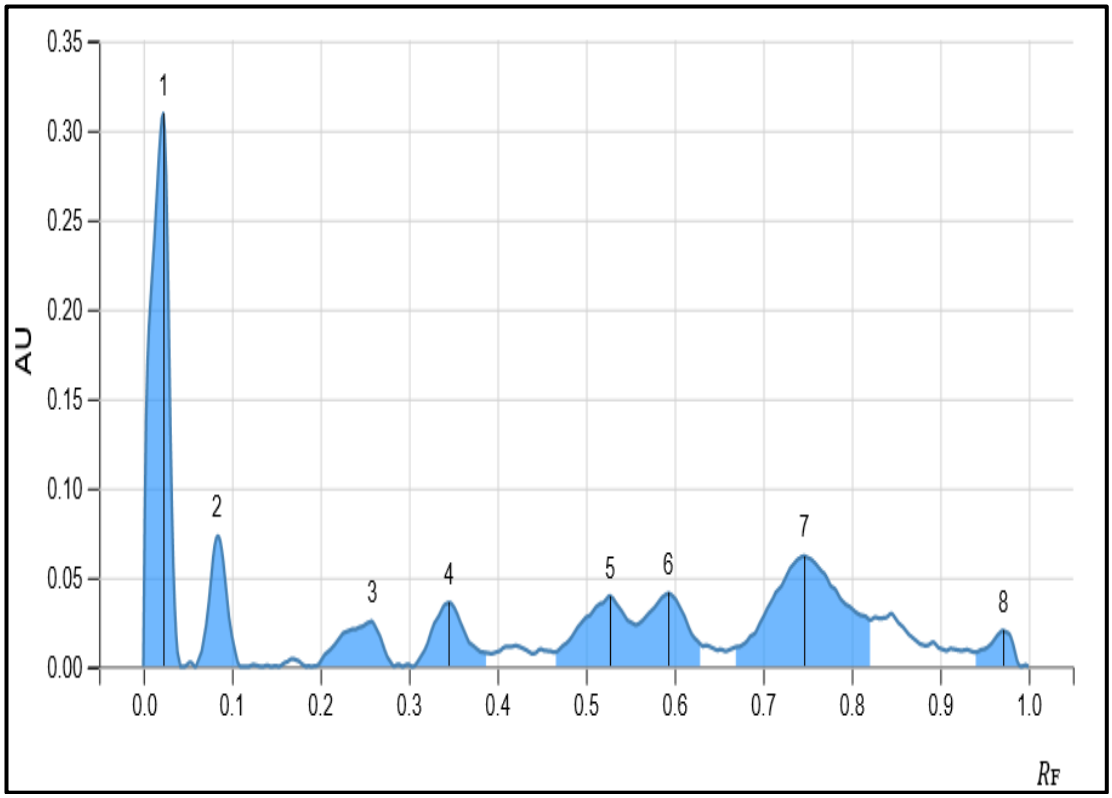


Fig 4.25**:** HPTLC chromatogram of MMSK12 SS1 performed at UV 254.

Table 4.15: R_F value and area percentage in HPTLC of SS1of MMSK12

Peak	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.023	0.3092	51.09	0.042	0.0000	0.00731	31.87
2	0.060	0.0000	0.084	0.0730	12.06	0.110	0.0000	0.00161	7.03
3	0.194	0.0000	0.258	0.0252	4.17	0.294	0.0000	0.00130	5.66
4	0.305	0.0000	0.345	0.0357	5.90	0.394	0.0071	0.00156	6.82
5	0.463	0.0080	0.527	0.0394	6.51	0.556	0.0232	0.00239	10.42
6	0.556	0.0232	0.594	0.0409	6.76	0.632	0.0114	0.00219	9.55
7	0.665	0.0099	0.747	0.0618	10.21	0.821	0.0261	0.00594	25.87
8	0.940	0.0079	0.971	0.0200	3.31	0.992	0.0000	0.00064	2.79

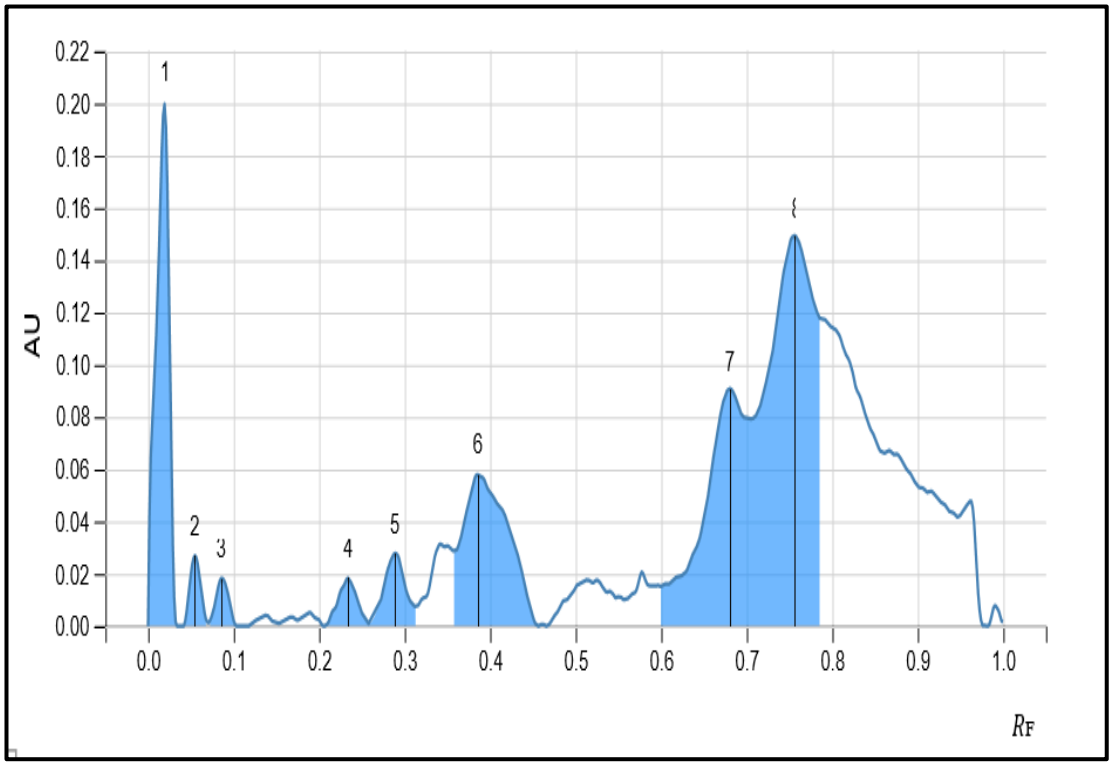


Fig 4.26**:** HPTLC chromatogram of MMSK12 SS2 performed at UV 254.

Table 4.16: R_F value and area percentage in HPTLC of SS2 OF MMSK12

Peak#	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.019	0.1998	33.92	0.034	0.0000	0.00353	14.60
2	0.042	0.0000	0.055	0.0269	4.57	0.069	0.0014	0.00039	1.63
3	0.071	0.0013	0.085	0.0182	3.09	0.103	0.0000	0.00031	1.30
4	0.206	0.0000	0.234	0.0183	3.11	0.260	0.0009	0.00047	1.93
5	0.260	0.0009	0.289	0.0278	4.71	0.313	0.0076	0.00080	3.32
6	0.358	0.0288	0.385	0.0579	9.83	0.456	0.0000	0.00359	14.82
7	0.600	0.0152	0.681	0.0908	15.42	0.705	0.0793	0.00531	21.95
8	0.705	0.0793	0.756	0.1494	25.36	0.787	0.1176	0.00979	40.45

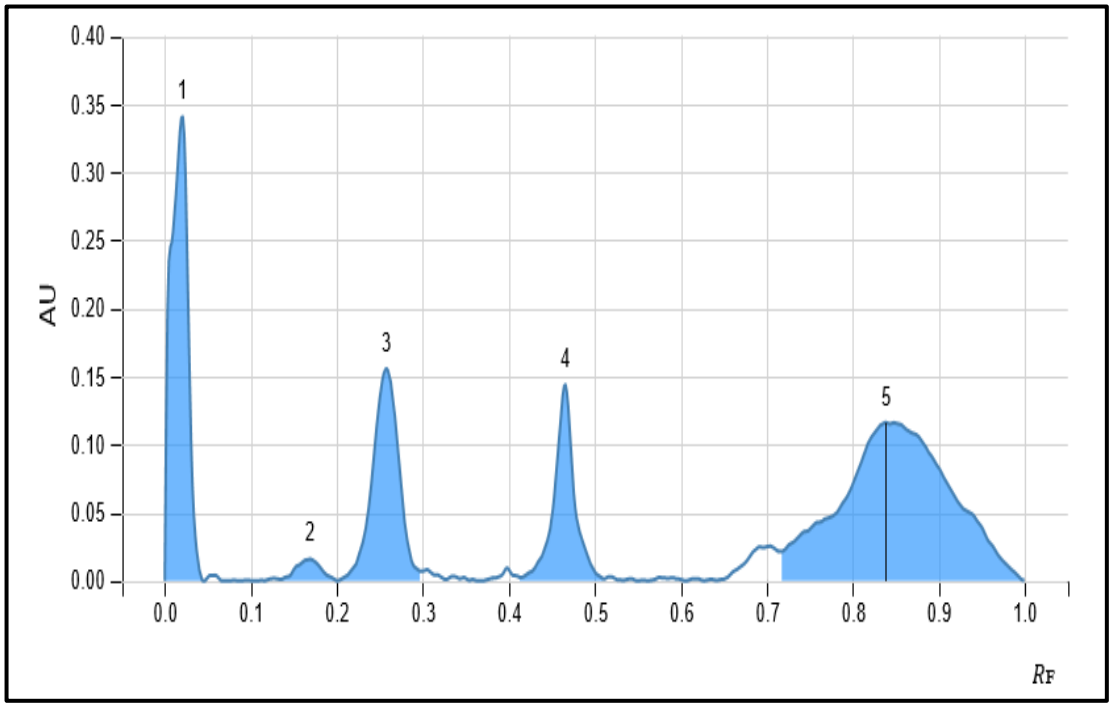


Fig 4.27**:** HPTLC chromatogram of MMSK12 SS3 performed at UV 254.

Table 4.17: R_F value and area percentage in HPTLC of SS3 of MMSK12

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.021	0.3406	44.09	0.045	0.0000	0.00807	22.71
2	0.135	0.0009	0.169	0.0159	2.05	0.200	0.0000	0.00053	1.50
3	0.202	0.0000	0.258	0.1558	20.17	0.298	0.0070	0.00553	15.56
4	0.413	0.0026	0.466	0.1440	18.64	0.511	0.0014	0.00388	10.91
5	0.718	0.0216	0.839	0.1162	15.04	1.000	0.0002	0.01753	49.31

CHAPTER 5

DISCUSSION

DISCUSSION

Microorganisms exhibit a remarkable and concerning ability to develop multi-drug resistance (MDR). This phenomenon arises when these organisms acquire and express multiple genetic mechanisms that enable them to evade the effects of various antimicrobial agents, including antibiotics and antifungals. In recent years, the prevalence of these MDR pathogens has surged, In recent times, the occurrence of these multidrug-resistant (MDR) pathogens has increased significantly, due to certain factors like the excessive and improper use of antibiotics in human healthcare and farming, along with insufficient measures for infection control. This alarming trend required the crucial need for the development of new antimicrobial agents that possess the capability to effectively target and eradicate resistant strains. Novel strategies may include the exploration of alternative therapeutic approaches, such as the use of bacteriophages, antimicrobial peptides, and combinatorial therapies that can overcome resistance mechanisms. Additionally, the implementation of robust surveillance systems and stewardship programs is crucial to monitor resistance patterns and promote responsible use of existing antibiotics. This proactive approach towards the development of innovative treatments and improved management frameworks is widely regarded as one of the most effective strategies for combating these formidable and evolving organisms, thereby safeguarding public health and ensuring the effectiveness of antimicrobial therapies for future generations. (Kenneth, 2009).

In response to this escalating challenge, there is a growing interest in harnessing natural products, particularly those derived from traditional medicinal plants. These plants are celebrated for their rich potential of biological properties and are emerging as vital sources for discovering novel bioactive compounds. The exploration of medicinal plants has gained considerable momentum, with many research initiatives focusing on their potential to yield anti-microbial compounds. Such compounds are crucial for the advancement of new classes of antibiotics that can effectively address the challenges posed by antibiotic resistance (Schultes, 1960).

Actinobacteria sourced from medicinal plants emerge as a unique and valuable reservoir of diverse bioactive compounds, which show significant potential for therapeutic applications. The chemical substances produced by these microorganisms

may be intricately linked to the specific traits and qualities of the host medicinal plant, suggesting a fascinating interplay between the bacteria and the plants they inhabit. This relationship not only enhances our understanding of natural medicine but also opens new avenues for discovering innovative treatments derived from nature's own pharmacy. The promising nature of our findings prompted us to embark on an exploration of the diverse traditional medicinal plants native to Mizoram, a region known for its rich botanical heritage. Our investigation focuses on understanding the complex community of endophytic actinobacteria that inhabit these plants, as well as assessing their biosynthetic potential. Specifically, we aim to evaluate how these microscopic organisms contribute to the production of compounds that exhibit strong antioxidant properties and antimicrobial activity, offering insights into their potential applications in modern medicine.

The five selected medicinal plants *Tithonia diversifolia, Centella asiatica* (L.) Urb., *Mikania micrantha, Mirabilis jalapa* L. and *Clerodendrum colebrookianum* Walp., are well known traditional medicinal plants of Mizoram. *Tithonia diversifolia*, commonly referred to as Bawngpu pangpar in Mizo, and popularly known as the Mexican sunflower, is a vibrant and striking plant that flourishes during the months of November and December. This extraordinary species is celebrated not only for its ornamental allure but also for its impressive array of medicinal properties. Research has identified a range of significant phytochemicals in *T. diversifolia*, including tagitinins, tirotundin, and flavones (Tona et al., 1999). Traditionally, local communities have harnessed the therapeutic potential of this plant to alleviate various ailments. It is particularly valued for its effectiveness in treating sore throats, and addressing respiratory issues (Soren and Lalthanpuii, 2021). In addition, local healers utilize the flower heads of *Tithonia diversifolia* for their remarkable ability to promote healing, applying them to treat wounds and bruises (Singh et al., 2017). Beyond the local context, studies conducted by researchers outside of Mizoram have revealed that this plant exhibits anti-inflammatory properties (Rüngeler et al., 1998) as well as anti-diarrhoeal effects (Tona et al., 1999), further showing its significance as a valuable resource in traditional medicine. *Mikania micrantha*, commonly known for its diverse medicinal properties, has a rich history of use in traditional medicine for treating a variety of ailments. Among its many applications, it is notably employed in the

management of diseases such as tuberculosis, skin disorders, fevers, and wounds, as well as more severe conditions like malaria (Sharma et al., 2001). In the region of Mizoram, local healers have utilized this remarkable plant for numerous years, particularly in the treatment of stomach cancer (Singh et al., 2017). The preparation of the medicinal decoction involves a meticulous process: the roots of *Mikania micrantha* are thoroughly cleaned and then simmered with the rhizomes of *Curcuma longa*, alongside its leaves, in water. This concoction is administered orally for effective healing (Sharma et al., 2001).

Furthermore, research conducted by various scholars has highlighted the plant's impressive range of therapeutic properties, illustrating its antibacterial (Okwori et al., 2007), wound healing, anti-inflammatory, antinociceptive, antipyretic (Okunade, 2002), and cytotoxic effects (Adebayo et al., 2010). The evidence gathered highlights *Mikania micrantha* as not only a vital component of traditional medicine but also as a subject of significant scientific interest globally. *Clerodendrum colebrookianum*, a vibrant and versatile plant, is widely consumed as a nutritious vegetable in the lush region of Mizoram. Beyond its culinary uses, the local populace harnesses its medicinal properties, particularly through the preparation of a decoction made from 4 to 5 fresh leaves, which is administered orally 2 to 3 times daily in the management of hypertension and diabetes. Additionally, for treating colics in infants, a potent leaf juice—5 mL, taken twice daily—demonstrates its effectiveness. This remarkable plant is also employed in traditional treatments for jaundice, as noted by Soren and Lalthanpuii in 2021. These practices vividly illustrate how the people of Mizoram have long relied on the wisdom of their ancestors to utilize this botanical resource in addressing various health issues, showcasing a deep connection to their natural environment and traditional healing knowledge.

In the present study, we successfully isolated 54 endophytic actinobacteria from five selected medicinal plants, each contributing uniquely to our findings. The plant *Mirabilis jalapa* emerged as the most prolific source, yielding 18 distinct isolates. Following closely was *Centella asiatica*, which provided 15 isolates, showcasing its rich microbial diversity. *Mikania micrantha* also proved significant, with 11 isolates, while *Tithonia diversifolia* contributed 6, and from *Clerodendrum colebrookianum* Walp, 4 isolates were obtained. The relative abundance of actinobacteria found in

Mirabilis jalapa, *Centella asiatica*, and *Mikania micrantha* may be attributed to their growing patterns, particularly their proximity to the soil. This closeness seems to foster a favourable environment for the development of endophytic communities. Among the 54 isolates we identified two best endophytic actinobacteria belonging to the genera Streptomyces, specifically *Streptomyces* sp. (MMSK12) and *Streptomyces* sp. (MMSK47). These two isolates are obtained from *Centella asiatica* and *Mirabilis jalapa*. *Centella asiatica*, widely known as gotu kola, is highly esteemed in Ayurvedic medicine for its extensive array of health benefits (Orhan 2012). This remarkable herb has been employed for centuries to treat various ailments, notably leprosy and asthma (Gohil et al., 2010). Beyond its historical uses, *Centella asiatica* is renowned for its potent healing properties, effectively promoting the recovery of ulcers and dermatitis while also demonstrating efficacy in treating skin tuberculosis and wounds (Park 2021). Furthermore, its therapeutic repertoire extends to alleviating arthritis, offering relief from joint inflammation and pain (Tan 2021). The herb plays a vital role in supporting vascular health, addressing issues like varicose veins and aiding in the regulation of elevated blood pressure (Ariffin et al., 2011). Its diverse therapeutic properties render it an indispensable herb in holistic health practices. Extensive research has shown a wide range of bioactivities associated with *Centella asiatica*, including impressive antioxidant, antimicrobial, neuroprotective, thrombolytic, and cytotoxic effects (Torbati et al., 2021; Park et al., 2017; Pittella et al., 2009). In the state of Mizoram, *Centella asiatica* holds a cherished place in traditional medicine. According to a study by Lalruatpuii et al. (2024), this remarkable herb has demonstrated effectiveness in treating various skin disorders, showcasing its therapeutic potential. Beyond its dermatological benefits, *Centella asiatica* is revered as a memory enhancer, often praised for its cognitive-stimulating properties. The lush green leaves of this plant are typically boiled, and the resulting infusion is consumed as a natural remedy for respiratory issues such as asthma, as well as for various eye ailments. Moreover, a decoction made from dried leaves serves as a valuable approach to managing hypertension, providing a holistic method for maintaining cardiovascular health. The freshly extracted juice of the leaves is celebrated for its detoxifying qualities, acting as a powerful agent for blood purification. Further emphasizing its diverse medicinal applications, research by Shankar and Rawat (2012) from Mizoram

highlights the practice of consuming 5 to 8 fresh leaves three times daily to relieve stomach aches and aid in the dissolution of kidney stones. Additionally, incorporating 5 to 10 fresh leaves each morning is believed to enhance vision, underscoring the multifaceted nature of this incredible herb. *Centella asiatica* stands as a testament to the rich heritage of plant-based healing that thrives in the vibrant culture of Mizoram. This makes *Centella asiatica* not only a valuable resource in traditional medicine but also a focal point of modern scientific inquiry into natural therapies.

Mirabilis jalapa, commonly known as the marvel of Peru, is renowned for its diverse array of pharmacological properties. This remarkable plant exhibits potent anti-inflammatory effects, as highlighted by Singh et al. (2010). Additionally, it is known for its powerful antioxidant capabilities, which have been documented in studies by Zachariah et al. (2011), Hajji et al. (2010), and Oladunmoye (2012). Furthermore, *Mirabilis jalapa* displays significant antibacterial properties, with research from Hajji et al. (2010) and Cammue et al. (1992) affirming its efficacy against various pathogens. This plant holds a cherished place in traditional medicine, particularly in the region of Mizoram, where it has been used for generations for its therapeutic benefits, as noted by Lalzarzovi and Lalramnghinglova (2016). According to researchers in Mizoram, the juice extracted from the rhizome is consumed 1 or 2 times orally in doses of 5-10 mL daily for its aphrodisiac properties. For the treatment of sexually transmitted diseases, a potent mixture of the rhizome with black pepper along with ginger is grind together and the juice of the mixture is administered orally at a dosage of 10 mL, four times a day. To alleviate skin irritations such as itches, the fresh juice from the leaves is applied topically. An infusion made from the leaves is used externally to help reduce swelling caused by bone fractures or sprains. Furthermore, a decoction derived from the entire plant is suggested for oral consumption 2-3 times in quantities of 10-15 mL daily, as a remedy for kidney and urinary infections. This decoction is also utilized as an effective diuretic (Sharma et al., 2001).

Colonization of actinobacteria initiates at the roots, where they thrive in the soil before being systematically transmitted upward along the stem, guided by the flow of essential nutrients. In our study, we investigate the potential endophytic actinobacteria isolated from traditional medicinal plants to uncover their bioactive compounds, particularly focusing on their antioxidant and antimicrobial properties. The selected

plants i.e. *Centella asiatica*, *Mikania micrantha*, *Mirabilis jalapa*, *Clerodendrum colebrookianum* Walp., and *Tithonia diversifolia*, all of which were sourced from Aizawl, Mizoram, situated within the northeastern region of India, a vibrant area recognized as part of the Indo-Burma biodiversity hotspot. Notably, other researchers have also contributed valuable findings by isolating endophytic actinobacteria from these same selected plants. For instance, Phuakjaiphaeo and Kunasakdakul (2015) successfully isolated 19 strains of actinobacteria from the stems of *Centella asiatica*. Similarly, Passari et al. (2020) identified 12 unique strains of endophytic actinobacteria from *Mirabilis jalapa*. In a separate study, Chagas et al. (2016) isolated endophytic *Streptomyces* sp. strain RTd22 from *Tithonia diversifolia*. Additionally, Momin and Tripathi (2018) reported the isolation of 7 actinobacteria strains from both the roots and stems of *Mikania micrantha*. These collectively underscore the rich potential of these medicinal plants in yielding valuable microbial strains with promising therapeutic applications.

Among the selected medicinal plants studied, *Mirabilis jalapa* L. yielded the highest number of microbial isolates (n=18). The majority of these isolates were extracted from the stems, while a smaller fraction was obtained from the delicate leaf fragments. The presence or absence of endophytic microbes within a specific tissue sample is intricately influenced by the internal composition of the tissue as well as the surrounding environmental conditions. Factors such as soil quality, climatic variables, geographic location, the particular plant species, age of the plant, and even the specific organ of the plant itself play significant roles in shaping the population and diversity of these microbial endophytes (Tan and Zou, 2001). A diverse array of five specialized nutritional media SCA (starch casein agar), ISP5 (glycerol asparagine agar base), ISP7 (tyrosine agar medium), AIA (actinomycetes isolation agar) and King's medium were employed to isolate various organisms due to the variability in nutrient uptake among different species. The results of the isolation process revealed that the ISP7 medium produced the highest number of isolates, supporting findings from previous studies conducted by Khirennas et al. (2023). This outcome highlights the effectiveness of ISP7 in nurturing a broader spectrum of microbial life, emphasizing its significance in isolation methodologies. The isolation of microbial strains was conducted employing a surface sterilization protocol, utilizing 70% ethanol and a 1% sodium hypochlorite

solution. This method is acknowledged for its ability to greatly diminish microbial contamination on surfaces. The application of 70% ethanol serves a dual purpose: not only does it rapidly penetrate microbial cell membranes, but it also denatures proteins, resulting in a substantial reduction in viable microbial counts. Specifically, this step can diminish microbial load by 3-5 log10 colony-forming units (CFU). In addition, the use of 1% sodium hypochlorite solution further enhances the decontamination process. Sodium hypochlorite acts as a strong oxidizing agent, disrupting various cellular components and leading to death of a wide range of pathogens. This step is capable of reducing microbial contamination by an additional 4-6 log10 CFU. When these two sterilization methods are combined, the cumulative effect can yield an impressive overall reduction in microbial contamination, achieving a decrease of 6-8 log10 CFU or potentially even more. This synergistic approach not only covers a broader spectrum of microorganisms—including bacteria, viruses, and fungi—but also provides a more prolonged antimicrobial effect. Such comprehensive sterilization protocols are crucial in laboratory settings and healthcare environments to ensure the integrity of microbial cultures and the safety of personnel. (Rutala and Weber, 2021). The surface sterilization technique employed in this study, referred to as the conventional method, has been demonstrated to be highly effective in previous research conducted by Waheeda and Shyam (2017) as well as Cao et al. (2004). Additionally, a comprehensive evaluation by Schulz et al. (1993), which tested seven various sterilization methods, further validated that the surface sterilization method applied in this study stands out as a reliable and efficient approach.

Actinobacteria are well known for their exceptional capability to produce a diverse number of bioactive compounds, which include antibiotics, immunosuppressive drugs, antitumor agents, plant growth hormones, as well as a variety of valuable biological substances such as enzymes, alkaloids, and vitamins. These compounds play a crucial role in the pharmaceutical industry, underscoring the significance of Actinobacteria in drug development and agricultural biotechnology (Uzma et al., 2018; Mishra et al., 2017; Wu et al., 2010).

In our current investigation, two particular strains, MMSK12 and MMSK47, emerged as the most promising candidates; they demonstrated a remarkable ability to inhibit 11 out of 15 tested pathogenic strains, with zones of inhibition varying from 4 mm to 16

mm in diameter in the secondary antimicrobial screening using antimicrobial diffusion assay. A crude extract with a concentration of 20 mg/mL was utilized for this assay, which is commonly regarded as a preliminary standard when evaluating the antimicrobial efficacy of natural products, extracts, or compounds against a variety of microorganisms. This concentration represents a well-established baseline that supports the integrity of our assessments. This specific concentration is strategically chosen to achieve a balance; it is sufficiently high to exert an inhibitory effect on microbial growth while remaining low enough to avoid cytotoxicity to nearby cells or tissues. Furthermore, 20 mg/mL is a widely recognized standard in the field, facilitating meaningful comparisons across diverse studies and experiments. This concentration also ensures an adequate quantity of the test substance available to interact with the microorganisms, thereby enhancing the probability of detecting notable antimicrobial activity. Our findings provide compelling support for the hypothesis that the medicinal properties of certain plants may be partially attributed to the presence of endophytes within their tissues (Strobel et al., 1998). The results indicate that endophytes have an important role in the production of medicinal compounds in certain plants and highlight the importance of considering the complex interactions between plants and their associated microorganisms in the search for novel medicinal compounds. Notably, the observed antagonistic activity against a range of tested bacterial pathogens paves the way for further investigations aiming at isolating potential antimicrobial compounds. These results align with previous research conducted by Aravamuthan et al. (2010), who successfully isolated actinobacteria from soil samples and demonstrated their efficacy against clinical multi-drug resistant (MDR) pathogens, thereby reinforcing the rationale of our current investigation. The same test was done by Singh et al., 2012 from actinobacteria isolated from the soil and found antimicrobial activity against three human pathogenic bacteria i.e. Vancomycin-Resistant *Enterococci* (VRE), Methicillin-Resistant *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Several researchers support the findings that endophytic actinobacteria, which are specialized bacteria residing within the tissues of various medicinal plants, particularly those with well-documented ethnobotanical uses, represent promising candidates for the extraction of potent antimicrobial natural products. These microorganisms have evolved alongside their host plants, potentially

offering unique bioactive compounds that might result in the development of novel therapeutic agents. The work of Sharma et al. (2001) revealed the significance of exploring these relationships as a means to unlock the pharmaceutical potential inherent in traditional herbal remedies. Furthermore, to support our research, Bhakyashree and Kannabiran (2020) have also successfully isolated the strain *Streptomyces* sp. VITBKA3, which demonstrates notable antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). This finding highlights the potential of *Streptomyces* sp. VITBKA3 is a valuable candidate for developing new antimicrobial agents in the fight against resistant bacterial infections.

To ascertain their taxonomic identity, these strains underwent rigorous analysis through 16S rRNA sequencing. The reliability of 16S rRNA sequencing for actinobacterial identification is notably high, yet it is influenced by various interdependent factors. The universal applicability of the 16S rRNA gene, coupled with its remarkable sensitivity and specificity, positions it as a difficult tool for the precise characterization of bacterial taxa. However, several limitations can compromise its accuracy, including challenges related to variable region selection, primer bias, database inadequacies, sequence ambiguity, and the potential for contamination. To enhance the reliability of this method, we employ a series of strategic practices, such as selecting multiple hypervariable regions for deeper resolution, optimizing primer selection to minimize bias, utilizing rigorous and high-quality reference databases, implementing strict quality control measures, and incorporating complementary identification methods. These efforts collectively enhance the reliability of actinobacterial identification and deepen our understanding of microbial diversity. The results from the BLAST analysis indicated that both MMSK12 and MMSK47 are classified within the genus Streptomyces, it is a group recognized for its production of antibiotics. The identification of MMSK12 and MMSK47 as belonging to the genus Streptomyces may provide insight into their notable antimicrobial properties. This is significant because Streptomyces is renowned for being one of the largest and most prolific producers of antibiotics among microbial species. Previous studies have consistently highlighted the remarkable capacity of this genus to generate various antimicrobial compounds (Watve et al., 2001; Atta, 2015). Streptomyces can produce a variety of antibiotics because of its unique genetic

structure. This unique genetic composition empowers the organism to synthesize secondary metabolites by a process called polyketide synthesis. During this procedure, simple molecular building blocks are intricately assembled and subsequently modified, allowing for the creation of elaborate and specialized molecules. This careful process leads to the creation of various strong antibiotic compounds. These compounds show how the organism has evolved to defend itself against harmful microbes.

A detailed morphology study performed with Scanning Electron Microscopy (SEM) revealed the complex structures of the aerial mycelia and spores for both MMSK12 and MMSK47. The findings align with those reported by Ullah et al. in 2012, which described the presence of branched and filamentous colonies, as well as chains of microspores with a notably smooth surface morphology. This morphological study reveals the complexity and diversity of the actinobacteria being examined. Scanning Electron Microscopy (SEM) is used for our analysis because of its exceptional resolution capabilities, which far surpass those of a conventional light microscope. For this investigation, we employed a magnification level of 15KX, allowing us to achieve a detailed view of the specimens under examination. To clarify, a magnification of 1,000X is equivalent to 1KX, highlighting the significant scale at which our observations were made.

These findings are significant because of the prevalence of resistant gram-positive bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA), which pose severe health threats and contribute to a range of clinical syndromes worldwide (Ippolito et al., 2010). Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a type of bacteria recognized for its ability to resist many antibiotics, such as methicillin and several penicillins. This pathogen has raised increasing concern across the globe due to its rising prevalence and its capacity to inflict serious infections. These infections can manifest in numerous forms, including painful skin and soft tissue infections, severe respiratory tract infections, potentially life-threatening bloodstream infections, and bone and joint infections. Transmission of MRSA often occurs through direct skin-to-skin contact, but it can also spread via contaminated surfaces or through healthcare-related interactions, posing a significant challenge in clinical settings. To effectively address MRSA infections, it is important to utilize strong antibiotics as part of the

treatment plan, such as vancomycin or linezolid, to effectively combat this resilient rival and prevent further complications. Awareness and prevention strategies are crucial in managing the risks associated with this emerging public health issue. While vancomycin remains the primary treatment option against MRSA, but the MRSA strains have adapted through mechanisms such as thickening their cell walls, altering their cell membrane structure, and producing enzymes that inactivate vancomycin. This resistance poses significant clinical challenges, including limited treatment options, increased risk of complications, and transmission risk, the alarming increase in less susceptible strains requires the exploration of alternative therapeutic possibilities to combat these tough pathogens effectively.

Present research has revealed that MMSK12 and MMSK47 may emerge as promising alternative therapeutic agents, particularly due to their remarkable ability to inhibit 11 strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from the 15 strains that were tested. This efficacy is highlighted by their impressive minimum inhibitory concentration (MIC) values, with MMSK12 exhibiting a MIC as low as 1 mg/mL and MMSK47 showing 2 mg/mL, both determined through plate-based and broth dilution methodologies. Using broth-based Minimum Inhibitory Concentration (MIC) testing is often more effective for high-throughput screening because it works well with automation. This helps to make the process much faster and easier. In contrast, plate-based methods are also employed for comparative analysis. One key advantage of plate-based testing lies in its instantaneous visualization, allowing for quick assessment of results. However, our findings indicate that the broth dilution method consistently yields better results, with lower MIC values. This suggests that the broth dilution technique is more efficient in providing precise measurements, as it produces results in decimal form, a capability that plate-based methods lack, where values are only reported as whole numbers.

The MIC findings strongly suggest that the metabolites produced by the MMSK12 and MMSK47 strains possess a broad spectrum of antimicrobial activity, which is crucial in the ongoing battle against multidrug-resistant *Staphylococcus aureus* (MRSA) pathogens—one of the most difficult challenges in modern healthcare. Furthermore, the research highlights the significant antimicrobial potential often associated with endophytic actinobacteria. MMSK12 and MMSK47 are identified as *Streptomyces* sp.

and *Streptomyces* species play a crucial role in combating Gram-positive pathogens through a variety of mechanisms. These remarkable actinobacteria produce numerous antimicrobial compounds that target essential bacterial functions. One of the primary methods employed by Streptomyces is the inhibition of cell wall synthesis, a vital process for bacterial integrity. For instance, well-known antibiotics such as streptomycin and tetracycline are synthesized by these organisms. These antibiotics effectively bind to the bacterial ribosome, obstructing protein synthesis and ultimately triggering cell death (Kohanski et al., 2010). In addition to targeting protein synthesis, *Streptomyces* species also produce compounds like daptomycin and telavancin. These agents interact directly with the bacterial cell membrane, inducing significant alterations to its structure and function, which can lead to cell lysis (Tran et al.,2015). This disruptive action on the membrane is essential, as it compromises the bacterial cell's protective barrier, rendering it vulnerable. Moreover, certain Streptomyces species secrete enzymes that specifically degrade the bacterial cell wall, such as lysozyme. This enzyme targets the peptidoglycan layer, a critical component of Gram-positive bacteria's structural framework, facilitating its breakdown (Fróes et al.,2012). The diverse number of antimicrobial compounds produced by Streptomyces species exemplifies their evolutionary adaptation and effectiveness in inhibiting the growth of Gram-positive pathogens, making them invaluable in the fight against bacterial infections.

Natural antioxidants play a crucial role in enhancing the antioxidant capacity of plasma, subsequently reducing the risk of various diseases (Okazaki et al., 1995). Synthetic antioxidants, despite their extensive application across various industries, present several noteworthy limitations. One prominent drawback is their inherent instability; these compounds can degrade rapidly under certain conditions, resulting in a loss of potency and diminishing their effectiveness over time. Additionally, when used in high concentrations, synthetic antioxidants can exhibit toxic properties. This could result in serious negative health impacts, such as harm to the liver and kidneys, along with an increased risk of cancer.

Moreover, these substances can disrupt crucial cellular signalling pathways, which may interfere with normal cellular functions and lead to a cascade of unintended consequences. This disruption can compromise the delicate balance within biological

systems, potentially jeopardizing overall health (Augustyniak et al., 2010). Furthermore, synthetic antioxidants frequently exhibit limited bioavailability and poor absorption, which can significantly reduce their capacity to penetrate target tissues and exert their beneficial effects at the cellular level (Carocho and Ferreira, 2013). Despite the known benefits of natural antioxidants, there has been limited research exploring the antioxidant properties of extracts derived from endophytic microorganisms. Thus, evaluating the antioxidant activity of these extracts has significant interest in the scientific community. Among the various methods available, the DPPH and ABTS+ assays stand out as traditional, rapid, and widely adopted techniques for assessing antioxidant activity. Antioxidants function as electron donors, effectively neutralizing free radicals such as DPPH and eliminating ABTS+ radicals by inhibiting the action of potassium persulfate (Re et al., 1999). In the present study, the methanolic crude extracts of the two strains, MMSK12 (*Streptomyces* sp.) and MMSK47 (*Streptomyces* sp.), exhibited a low activity against the DPPH radical, yet demonstrated high effectiveness against the ABTS radical. It is essential to highlight that both ABTS and DPPH radicals are structurally different, this difference leads to varying reactivities. For instance, certain organic compounds, particularly dihydrochalcones and flavanones, do not engage with DPPH radicals; however, they readily interact with ABTS radicals (Platzer et al., 2021). This divergence in structure may account for the differences observed in the IC50 values reflecting the radical scavenging activities of MMSK12 and MMSK47 against ABTS+ and DPPH radicals.

In 2004, Molyneux developed a classification system for antioxidant activity based on IC50 values, providing a framework to assess the potency of various compounds. According to this classification, compounds exhibiting IC50 values of less than 0.05 mg/mL are characterized as highly active, while those with values that ranges from 0.05 to 0.1 mg/mL are categorized as active. Furthermore, compounds with IC50 values between 0.1 and 0.15 mg/mL are classified as demonstrating medium activity, and those with values from 0.151 to 0.2 mg/mL are considered weak in their scavenging abilities. Recent findings indicate that the extract in question showed a significant ABTS scavenging activity, emphasizing its possibilities as a valuable source of natural antioxidants. The IC50 value of MMSK12 is measured at 82 µg/mL, while MMSK47 displays an IC50 value of 94.14 µg/mL. Both values fall within the

active category established by Molyneux, suggesting a robust level of antioxidant capacity. To support our finding that *Streptomyces* sp. has a high ABTS scavenging activity Tan et al., 2017 has found that *Streptomyces* sp. MUM212 has ABTS activity of 61,52% at 4mg/mL of the extract. In contrast, research by Kaur et al. (2017) revealed higher ABTS IC50 values for three distinct *Streptomyces* sp. strains: TEAE at 121.51 µg/mL, OCE at 352.48 µg/mL, and TCE at 354.24 µg/mL. These comparative results illuminate the considerable antioxidant potential of MMSK12 and MMSK47, positioning them as significant candidates for further research and development within the realm of antioxidant applications.

Phenolic compounds and flavonoids play an important role in the prevention of lipid oxidation, significantly contributing to antioxidant effects, as evidenced by various studies (Yin et al., 2007). The remarkable antioxidant potential of the extracts is primarily attributed to their rich phenolic content. In this present study, the Total Phenolic Content of MMSK12 (a strain of *Streptomyces* sp.) was found to be a noteworthy 68.6±0.51 mg of Gallic Acid Equivalent (GAE) per gram of extract. Similarly, MMSK47 (*Streptomyces* sp.) exhibited a substantial phenolic content of 60.4±0.77 mg of GAE per gram of extract. Both values significantly surpass the previously reported total phenolic content of endophytic *Streptomyces* sp. documented by Akshatha et al. in 2016, which measured a mere 2.56±0.82 µg GAE/g. In contrast, the total flavonoid content of CENK12 (*Streptomyces* sp.) was comparatively lower, recorded at 10.92±0.64 mg of Quercetin (QE) per gram, highlighting a distinct difference when compared to its total phenolic content.

The phenolic content in endophytes is largely a reflection of their remarkable potential to synthesize secondary metabolites, which serve as a vital defence mechanism against a variety of environmental stresses, pathogens, and insect predation. This biochemical ability allows endophytes to thrive in challenging conditions. Moreover, these microorganisms have evolved to produce phenolic compounds not only as a form of protection but also as a means of communication and interaction with their host plants, developing mutualistic relationships that benefit both parties. The levels of phenols within endophytes are further influenced by various factors, including the genetic makeup of the host plant, specific environmental conditions, and the presence of neighbouring microorganisms, all of which contribute to the dynamic interplay

between endophytes and their ecological niches. The flavonoid content produced by endophytic actinobacteria is also thought to be vital in the symbiotic relationships with their host plants, offering essential protection against pathogens and various environmental stresses. To assess the total flavonoid content, researchers have employed the colorimetric aluminium chloride ($AlCl_3$) method, which is the most widely recognized technique in this field. This method has been utilized in studies by various researchers, including Shabira et al. (2022), Passari et al. (2017), and Nafis et al. (2018), demonstrating its effectiveness in quantifying flavonoid levels in endophytic actinobacteria.

In this study, a comprehensive analysis of the methanolic extracts from two distinct strains of *Streptomyces* sp., namely MMSK12 and MMSK47 was conducted, both of which are recognized for their potent antimicrobial and antioxidant properties. The analysis employed Gas Chromatography-Mass Spectrometry (GC-MS), a commonly acknowledged analytical method renowned for its capability to detect and identify volatile organic compounds (VOCs). The analysis can reveal a presence of phenolic substances, including flavonoids and phenolic acids, terpenoids including monoterpenes and sesquiterpenes, and various alkaloids known for their pharmacological importance. These substances are valued for their strong antioxidant, anti-inflammatory, and antimicrobial characteristics, which play a significant role in their ability to address oxidative stress, inflammation, and infections. The gas chromatography-mass spectrometry (GC-MS) analysis provided a detailed and comprehensive understanding of the chemical composition of the crude extract, allowing for the identification of specific bioactive constituents. This informative baseline not only highlights the extract's potential health benefits but also lays the groundwork for further targeted isolation and characterization of these bioactive compounds, facilitating a deeper exploration into their ways in which they function and their possible uses in healthcare and industry. This method has been extensively utilized by researchers in various fields to accurately identify the presence of VOCs in complex samples (Jog et al., 2014; Bajo-Fernández et al., 2024). *Streptomyces* sp. is particularly well known for its ability to synthesize a number of secondary metabolites, many of which exhibit significant antimicrobial and antioxidant activities. The primary objective of our analysis was to identify the volatile components contained within the

methanolic crude extracts of MMSK12 and MMSK47. To identify the specific compound present in these extracts, we compared their retention times and relative retention factors against established authentic standards. The quantification of the compounds was carried out by evaluating their percentage composition based on peak areas, utilizing area normalization techniques. This method not only improves the dependability of our results but also offers important insights into the chemical variety of these extracts. The volatile compounds identified in the crude extract of MMSK12 and MMSK47 were analysed and compared against the extensive NIST17 online library, version 2.339. The NIST (National Institute of Standards and Technology) library serves as a vital resource, presenting a comprehensive database for the identification of compounds within Gas Chromatography-Mass Spectrometry (GC-MS) analysis. By comparing the mass spectrum of an unknown compound with the vast array of mass spectra from confirmed compounds in this repository, researchers can swiftly and accurately pinpoint the nature of the substance in question. This large library utilizes an efficient probability-based matching algorithm to calculate a sureness level for each identified match, effectively guiding researchers in their analysis. While the advantages of the NIST library are manifold offering rapid identification, exceptional accuracy, and extensive coverage of diverse compounds, it is not without its limitations. Challenges such as database constraints, variability in spectral data, and the requirement for interpretation by an analyst emphasize the importance of expertise in navigating the complexities of compound identification.

The analysis of MMSK12 uncovered 20 volatile organic compounds, among which several noteworthy substances were identified for their significant properties. Key compounds such as N-Decanoic acid (Yang et al., 2023; Ravichandiran et al., 2019), 1,4-Naphthoquinone(Wu et al., 2020; Huang et al.,2011), 2-hexanoic acid (Aranega-Bou et al., 2014; González et al., 2021), Anthracene (Kim et al., 2009; Popiołek 2021), Acetohydrazine (Boulebd et al., 2022; Küçük et al.,2011), 1,3-Dioxolane (Raskildina et al., 2024; Wood and Szewczak, 2007), and 3-Octanal (Matos et al., 2019; Desai et al., 2013) were found to exhibit remarkable antimicrobial and antioxidant capabilities, with some even displaying both beneficial properties simultaneously. This discovery suggests a compelling hypothesis: the antibacterial and antioxidant effects attributed to the extract may arise from the synergistic interactions of these bioactive compounds

to improve overall effectiveness. In the detailed analysis of MMSK47, a total of 19 volatile organic compounds were identified, revealing a diverse array of chemical components. Among these, notable substances such as Anthracene and decanoic acid have been cited in earlier literature (Kitahara et al., 2004; Shen et al., 2021) for their remarkable antimicrobial properties. Additionally, Octanal (Liu et al., 2012), Hydrazine (Popiolek, 2021), and various Dioxolanes (Kucuk et al., 2011; Ovsyannikova et al., 2013) were also detected, many of which are recognized for their potential antibacterial effects. Research has shown that anthracene possesses antimicrobial capabilities, as evidenced by studies done by Debbab et al. in 2012 and Dardeer et al. in 2020. Furthermore, a significant investigation into perylene's antibacterial activity demonstrated its effectiveness against *Mycobacterium tuberculosis* and Methicillin-resistant *Staphylococcus aureus* (MRSA) in a study by Yilmaz et al., 2023. Supporting our current research, additional studies on perylene's antimicrobial properties were undertaken by Rostaminejad et al., 2024, highlighting its relevance and potential in antimicrobial applications.

High Performance Thin Layer Chromatography (HPTLC) fingerprint analysis is a conventional and cost-effective technique for the analysis, separation, and qualitative identification of compounds. In this study, we utilized an optimized solvent system to detect anthracene, phenols and glycosides to which are primarily antioxidant and antimicrobial compounds in the methanolic crude extract of MMSK12 (*Streptomyces* sp.) and MMSK 47(*Streptomyces* sp.). in MMSK12 methanolic crude extract, eight peaks in both the phenol and glycoside solvent systems and 5 peaks in anthracene solvent system. Whereas in MMSK47, 5 peaks were observed in anthracene and glycosides solvent system and 7 peaks in phenols. This clearly suggest the presence of different antimicrobial and antioxidant components in both the methanolic crude extract of MMSK12 and MMSK47. This research will facilitate the quantitative determination of the compounds present in the methanolic extract of *Streptomyces* sp. In the research conducted by Shekar et al. in 2016, the ethyl acetate extract of *Streptomyces* sp. strain SFA5 was analysed using High-Performance Thin-Layer Chromatography (HPTLC) to identify the presence of antimicrobial compounds. The HPTLC analysis revealed distinct antimicrobial activities, indicated by Rf values of 0.38 and 0.46. These findings support our findings that the extract contains multiple

bioactive compounds, which may have the potential for further development as natural antimicrobial agents. The study emphasizes the importance of *Streptomyces* sp. as a promising source for discovering novel pharmacological substances. High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting analysis serves as an invaluable tool for the comprehensive characterization of crude extracts, offering a distinctive visual representation of their intricate chemical composition. This technique separates different parts of a crude extract based on their polarity and how much they stick to the stationary phase. The result is a chromatogram that acts like a unique fingerprint for the extract, showing its specific characteristics.

HPTLC fingerprinting has numerous advantages, including rapid analysis and exceptional resolution, making it a preferred method for authenticating samples and detecting any potential adulteration. This technique is extensively employed in quality control processes, thorough phytochemical analysis, and the systematic comparison of crude extracts. Key elements of interpretation include peak identification, quantification, and the comparison of fingerprints, all of which contribute to understanding the chemical landscape of the extracts in question.

Although phytochemical constituents are often overlooked in the exploration of the biological properties of actinobacteria, this research aims to emphasize the significant role these chemical compounds play in the interactions between plants and their endophytes. Phytochemicals are bioactive compounds including phenolic acids, flavonoids, alkaloids, and terpenoids, are primarily responsible for the antioxidant and antimicrobial properties observed in many plant species. Intriguingly, these compounds are also synthesized by the endophytes that inhabit these plants, creating a symbiotic relationship that may enhance the host plant's innate abilities to combat pathogens, inhibit microbial growth, and mitigate oxidative stress.

To strengthen the connection between the chemical constituents of the host plant and its associated endophytes, recent research conducted by Desai et al. (2013) has provided valuable insights. Their findings reveal that the host plant *Centella asiatica,* known for its traditional medicinal uses, contains significant levels of phenolic compounds and flavonoids, which contribute to its therapeutic effects. Similarly,

Mirabilis jalapa, renowned for its ornamental value and medicinal properties has shown a rich profile of these phytochemicals.

In a more comprehensive examination, the current study identifies that the endophytic strains of *Streptomyces* sp., specifically MMSK12 and MMSK47, which reside within the tissues of both *Centella asiatica* and *Mirabilis jalapa*, exhibit a similar chemical composition that is abundant in phenolic compounds and flavonoids. These bioactive molecules are well-known for their antioxidant properties, which play a critical role in the defence mechanisms of plants against oxidative stress and various environmental stressors. The shared chemical profile not only suggests a mutualistic relationship in which the endophytes benefit from the nutritional and chemical resources provided by the host plants but also raises intriguing questions regarding how this symbiosis could enhance the antioxidant capabilities observed in both organisms. The interaction between the endophytes and their host plants may be crucial for optimizing the synthesis and accumulation of these beneficial compounds, ultimately contributing to a higher resilience against pathogens, pests, and adverse environmental conditions. Moreover, the presence of these phenolic and flavonoid compounds in both the plant and its endophytes could elucidate their collective capacity to maintain plant health and vigour. This highlights a fascinating area of research that permits further investigation into the intricate dynamics of plant-endophyte interactions. Understanding these dynamics could have significant implications for agricultural practices and the development of medicinal applications, particularly in enhancing crop resilience and finding novel sources of natural antioxidants.

In the present investigation, we have employed crude extracts for our analysis because of their ability to encompass a wide number of bioactive compounds, which reflects the essential complexity and variability of natural substances found in various ecosystems. Utilizing these crude extracts allows us to explore the synergistic effects of multiple compounds acting simultaneously, offering insights into their collective biological activities. However, this approach is not without its challenges. One significant drawback is the difficulty in pinpointing which specific compound or combination of compounds is primarily responsible for the observed biological effects. This lack of specificity can complicate the interpretation of results and hinder the

development of targeted therapies. Therefore, the primary objective of this study is to establish a strong foundation for future research aimed at the isolation and purification of individual bioactive compounds. To this end, we are considering High-Performance Thin-Layer Chromatography (HPTLC) as a promising and effective methodology, which not only facilitates the separation of complex mixtures but also enables the identification of compounds based on their distinct chromatographic profiles. This approach may eventually lead to a more in-depth understanding of the mechanisms by which these compounds exert their biological activities, paving the way for advancements in pharmacology and natural product development.

CHAPTER 6

SUMMARY AND CONCLUSION

SUMMARY

- *Tithonia diversifolia* (Hemsl), *Centella asiatica* (L.) Urb., *Mikania micrantha* K, *Mirabilis jalapa* L. and *Clerodendrum colebrookianum* Walp., were the five selected traditional plants to isolate endophytic actinobacteria. The plants were collected in and around Aizawl, Mizoram, India.
- 54 distinct actinobacterial isolates were successfully isolated from the five selected traditional medicinal plants.
- The media employed included tyrosine agar medium (ISP7), actinomycetes isolation agar (AIA), starch casein agar (SCA), glycerol asparagine agar base (ISP5), and King's medium. The highest levels of actinobacterial growth were observed in ISP7, where 20 isolates were obtained from the media. The growth levels were subsequently followed by SCA, AIA, ISP5, and King's medium.
- Among the five medicinal plants selected *Mirabilis jalapa* L. yielded the greatest number of isolates (n=18), This was closely followed by *Centella asiatica* (L.) Urb, which contributed 15 isolates and *Mikania micrantha* K with 11 isolates, *Tithonia diversifolia* (Hemsl) with 6 isolates, and *Clerodendrum colebrookianum* Walp with 4 isolates
- All the 54 isolates were subjected to Primary Antimicrobial screening against 33 clinical MDR pathogens. 32 out of 54 isolates have shown inhibitory effects against three or more of the tested pathogens.
- From the 32 positive isolates from primary antimicrobial screening, 15 isolates with the best inhibition zone were selected for secondary antimicrobial screening
- 15 selected actinobacterial isolates were subjected to extract preparation and were used for secondary antimicrobial screening. For secondary antimicrobial screening, 20 mg/mL of the crude extract was tested against 15 clinical MRSA pathogens. All of the isolates have antimicrobial properties against at least to 6 of the tested pathogens.

- The best two isolates from secondary antimicrobial screening were selected i.e. MMSK12 and MMSK47 since the zone of inhibition for these isolates varied significantly, ranging from 4mm to16mm in diameter.
- The molecular characterization using 16S rRNA sequencing revealed that both MMSK 12 and MMSK47 are *Streptomyces* sp. The sequences obtained from these molecular identifications have been deposited in the NCBI GenBank database, with the respective accession numbers assigned as OR896092 for MMSK47 and OR896079 for MMSK12.
- Scanning electron microscope unveiled the intricate details of their spore surfaces, which appeared notably smooth and were adorned with branched, filamentous structures for both MMSK12 and MMSK47. Additionally, these microorganisms exhibited the formation of microspore chains, creating unique and complex colonies that captivated the eye with their elaborate arrangement.
- The minimum inhibitory concentration (MIC) zone of inhibition for MMSK12 was recorded at 1 mg/mL against 365 pathogen, 2 mg/mL against two pathogens (924 and 1246), while it was observed at 4mg/mL for pathogen 1098 and 12 mg/mL for 790 pathogen. In contrast, the MIC zone of inhibition for MMSK47 was found at 1 mg/mL for pathogens 365, 924, 1098, and 1246, and for pathogen 790, it was noted at 2 mg/mL.
- Similarly, the minimum inhibitory concentration (MIC) of the methanolic crude extract from MMSK12 and MMSK47 was evaluated using broth microdilution method. The EC_{50} value for MMSK12 2mg/mL against pathogen 924, 1098, and 1246, 5mg/mL for pathogen 365 and 6mg/mL for pathogen 790. The EC_{50} value for MMSK47 is 1mg/mL for pathogens 365, 924, 1098, and 1246 and 2mg/mL for pathogen 790.
- Antioxidant assays such as ABTS and DPPH were done for both MMSK12 and MMSK47. IC_{50} in ABTS assay for MMSK12 and MMSK47 are 82.78 µg/mL and 94.14 µg/mL, respectively. Whereas

the IC_{50} in DPPH assay is much lower as compared to the IC_{50} of ABTS with 668.3 µg/mL and 863.7 µg/mL respectively for MMSK12 and MMSK47.

- The total phenolic content and total flavonoid content was measured using UV-Vis Spectroscopy. The flavonoid content was comparatively lower to the phenolic content for both MMSK12 and MMSK47. The results of TPC analysis indicated that the MMSK12 extract contains a concentration of 68.6±0.51 mg of Gallic Acid Equivalent (GAE) per gram, while the MMSK47 extract has a slightly lower but still significant level of 60.4±0.77 mg of GAE per gram. Whereas, the results of TFC analysis was recorded at 10.92±0.64 mg of Quercetin (QE) per gram of the extract for MMSK12 and 12.89±0.73 mg of Quercetin (QE) per gram for MMSK47.
- 20 volatile organic compounds were detected in the crude extract of MMSK12 whereas, 19 volatile organic compounds were detected in the crude extract of MMSk47 in GC-MS analysis.
- For HPTLC, three optimized solvent system namely ethyl acetate: methanol: water (20:3:2), cyclohexane: ethyl acetate: formic acid (4:6:1) and toluene: ethyl acetate: methanol: acetic acid (3:5:1:0.5) to detect glycosides (SS1), phenols (SS2) and anthracene (SS3) respectively were used in HPTLC analysis. For MMSK47, 5 peaks were recorded in SS1 and SS3 whereas, 7 peaks were recorded in SS2 at 254nm. For MMSK12, 8 peaks were recorded in SS1 and SS2 and 5 peaks were recorded for SS3 at 254nm.

CONCLUSION

Microorganisms and plants are indispensable for sustaining life on Earth, forming the foundation of our ecosystems. They are integral to natural processes such as biogeochemical cycles, including the nitrogen and carbon cycles, which regulate nutrient flow and energy transfer. These organisms help preserve diverse ecological environments, fostering unique habitats that support specific plant and animal species, each adapted to their particular ecological niche. Key roles of microorganisms and plants include the generation of oxygen through photosynthesis, a process that not only supports aerobic life forms but also influences climate regulation. Additionally, they absorb carbon dioxide, thereby playing a crucial part in mitigating climate change. Beyond these roles, plants are vital for the synthesis of organic compounds—these compounds serve as food for humans and animals, as well as raw materials for medicines that treat a myriad of health conditions. Ethnomedicinal plants, which have been utilized for centuries in various cultures to address health issues, face a significant challenge due to their limited availability and accessibility. Many of these species are threatened due to habitat loss, overharvesting, and changing climate conditions. In this context, endophytic microorganisms that live within plant tissues without causing harm can be a promising pathway for research and development. Endophytic actinobacteria, in particular, has a potential in enhancing plant health and productivity while simultaneously producing bioactive compounds that can have medicinal properties. By exploring the relationships between these microbes and their host plants, researchers can discover valuable insights into their biology and chemistry. This knowledge can pave the way for innovative and safe applications that promote human health, increase agricultural productivity, and contribute to environmental sustainability. Ultimately, the unique capabilities of endophytes can help address the issues posed by the scarcity of ethnomedicinal plants and advance our understanding of natural resource management.

The urgent need for discovering and developing new medications has become more critical due to the issues related to treating and managing diseases caused by multiple drug-resistant (MDR) strains of MRSA. The unique and diverse endophytic actinobacteria found in unexplored environments present exceptional opportunities for discovering drugs and therapeutic agents. In addition to their medical applications, the

bioactive compounds produced by endophytic actinobacteria are beneficial for agricultural purposes, as they can enhance plant growth, protect against phytopathogens, insects, and pests, improve nutrient absorption, and help sustain crop productivity during extreme abiotic stress conditions such as salinity, drought, and waterlogging.

The current research has revealed that two strains of Streptomyces, designated as MMSK12 and MMSK47, have been successfully isolated from the traditional medicinal plants *Centella asiatica* and *Mirabilis jalapa*. These plants are indigenous to Mizoram, a region recognized as part of the Indo-Burma biodiversity hotspot, characterized by its rich variety of flora and fauna. Both MMSK12 and MMSK47 have demonstrated significant antimicrobial activity against clinical multidrug-resistant *Staphylococcus aureus* (MRSA), which poses a critical challenge in treating bacterial infections due to its resistance to many conventional antibiotics. Aside from their ability to combat microbes, MMSK12 and MMSK47 have also exhibited substantial antioxidant activities, suggesting their potential utility in combating oxidative stress-related disorders. Preliminary analysis using gas chromatography-mass spectrometry (GC-MS) have identified many bioactive compounds found in the methanolic crude extract of these strains. However, further investigations are needed to isolate, identify, and quantify the specific bioactive compounds that contribute to the observed antimicrobial and antioxidant effects.

The promising findings associated with *Streptomyces* sp. MMSK12 and MMSK47 imply that these strains could be invaluable sources for the extraction of bioactive compounds. Such compounds have significant implications for the pharmaceutical industry, particularly in the development of novel treatments for infections and diseases linked to oxidative stress. With targeted research and development, there is great potential for these strains to contribute to new therapeutic solutions.

REFERENCES

Adebayo AH, Tan NH, Akindahunsi AA, Zeng GZ, Zhang YM. (2010). Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L. (Asteraceae). *Pharmacogn Mag*., 6(21):62-6.

Akshatha JV, Prakash HS, Nalini MS. (2016), Actinomycete Endophytes from the Ethno Medicinal Plants of Southern India: Antioxidant Activity and Characterization Studies. J Biol Active Prod Nat 6(2): 166–172.

Al-Ansari M, Alkubaisi N, Vijayaragavan P, Murugan K. (2019). Antimicrobial potential of *Streptomyces* sp. to the Gram positive and Gram negative pathogens. *J infect public health*, 12(6):861-866.

Alexander BD, Johnson MD, Pfeiffer CD, et al. (2013). Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin. Infect. Dis*., 56:1724–1732

Alexander DC, Rock J, He X, Brian P, Miao V, Baltz RH. (2010). Development of a genetic system for combinatorial biosynthesis of lipopeptides in *Streptomyces fradiae* and heterologous expression of the A54145 biosynthesis gene cluster. *Appl. Environ. Microbiol.* 76:6877–6887.

Al-Rubaye AF, Hameed IH, Kadhim MJ. (2017). A review: uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. *Int J Toxicol Pharmacol Res*, 9(1):81-85.

Al-rubaye TS, Risan MH, Al-Rubaye D. (2020). Gas chromatography-mass-spectroscopy analysis of bioactive compounds from *Streptomyces* spp. isolated from Tigris river sediments in Baghdad city. *J Biotechnol Res Center*, 14(1):63-71.

Andreote FD, Gumiere T, Durrer A. (2014). Exploring interactions of plant microbiomes. Sci. Agric., 71:528–539.

Aranega-Bou P, de la O Leyva M, Finiti I, García-Agustín P, González-Bosch C. (2014). Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Front Plant Sci*, 5: 488.

Aravamuthan N, Gaurav Kumar, Karthik L, Bhaskara Rao KV. (2010). In vitro antagonistic activity of soil actinobacteria against multi drug resistant bacteria. *Pharmacologyonline*, 2:507-516.

Ariffin F, Heong Chew S, Bhupinder K, Karim AA, Huda N. (2011). Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. *J Sci Food Agric*, 91(15): 2731-9.

Atta HM. (2015). Biochemical studies on antibiotic production from *Streptomyces* sp.: Taxonomy, fermentation, isolation and biological properties. *J Saudi Chem Soc*, 19(1):12–22.

Augustyniak A, Bartosz G, Čipak A, Duburs G, Horáková LU, Łuczaj W, Žarković N. (2010). Natural and synthetic antioxidants: an updated overview. *Free radic res.*, 44(10), 1216-1262.

Bacon CW, White Jr JF. (2000). Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. In *Microbial endophytes*. CRC Press, 251-276

Bajo-Fernández M, Souza-Silva ÉA, Barbas C, Rey-Stolle MF, García A. (2024). GC-MS-based metabolomics of volatile organic compounds in exhaled breath: Applications in health and disease. A review. *Front Mol Biosci*., 10:1295955.

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-Kolthoff JP, et al. (2016). Taxonomy, physiology, and natural products of actinobacteria. *Microbiol. Mol. Biol. Rev*., 80 (1):1–43.

Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, et al. (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3 (2). *Nature* 417:141–147.

Berdy J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antibiot*., 65:385–395

Bérdy, J. (2005). Bioactive microbial metabolites. J Antibiot., 58(1):1-26.

Bhakyashree K, Kannabiran K. (2020). Actinomycetes mediated targeting of drug resistant MRSA pathogens. *Journal of King Saud University-Science*, 32(1):260-264.

Bogas AC, Ferreira AJ, Araújo WL, Astolfi-Filho S, Kitajima EW, Lacava PT, et al. (2015). Endophytic bacterial diversity in the phyllosphere of Amazon *Paullinia cupana* associated with asymptomatic and symptomatic anthracnose. Springer Plus, 4:1–13.

Bonnet M, Lagier JC, Raoult D, Khelaifia S. (2019). Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes. New Infect*., 34:100622.

Boucher HW, Corey GR. (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.*, 46:S344–S349.

Boulebd H, Zine Y, Khodja IA, Mermer A, Demir A, Debache A. (2022). Synthesis and radical scavenging activity of new phenolic hydrazone/hydrazide derivatives: Experimental and theoretical studies. *J Mol Struct.*, 1249:131546.

Boyanova L. (2005). Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods. *J Med Microbiol* 54(5):481–483.

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A. (2014). Metabolic potential of endophytic bacteria. *Curr. Opin. Biotech*., 27:30–37.

Brinkac L, Voorhies A, Gomez A, Nelson KE. (2017). The threat of antimicrobial resistance on the human microbiome. *Microbial ecology*, 74(4):1001-1008.

Cammue BP, De Bolle MF, Terras FR, Proost P, Van Damme J, Rees SB, Broekaert WF. (1992). Isolation and characterization of a novel class of plant

antimicrobial peptides form *Mirabilis jalapa* L. seeds. *J Biol Chem*., 267(4):2228–2233.

Campisano A, Antonielli L, Pancher M, Yousaf S, Pindo M, Pertot I. (2014). Bacterial endophytic communities in the Grapevine depend on pest management. *PLoS One* 9:e112763.

Cao L, Qiu Z, You J, Tan H, Zhou S. (2004). Isolation and characterization of endophytic Streptomyces strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. *Lett appl microbiol.*, 39(5):425-430.

Carocho M, Ferreira IC. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food chem toxicol.*, 51:15-25.

Castillo UF, Strobel GA, Mullenberg K, Condron MM, Teplow DB, Folgiano V et al. (2006). Munumbicins E-4 and E-5: novel broad-spectrum antibiotics from Streptomyces NRRL 3052. *FEMS Microbiol Lett,* 255:296–300

Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2019. US Department of Health and Human Services, Centres for Disease Control and Prevention (2019).

Chagas FO, Ruzzini AC, Bacha LV, Samborskyy M, Conti R, Pessotti RC, Pupo MT. (2016). Genome sequence of *Streptomyces* sp. strain RTd22, an endophyte of the Mexican sunflower. *Genome Announcements.*, 4(4), 10-1128.

Chandra P, Sharma RK, Arora DS. (2020). Antioxidant compounds from microbial sources: A review. *Food Res. Int.*, 129:108849.

Chater KF. (2016). Recent advances in understanding Streptomyces. *F1000Research, 5*: 2795.

Chitra G, Franklin DS, Sudarsan S, Sakthivel M, Guhanathan S. (2017). Indole-3-acetic acid/diol based pH-sensitive biological macromolecule for antibacterial, antifungal and antioxidant applications. *Int j biol macromol.*, 95:363-375.

Choo EJ, Chambers HF. (2016). Treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Infect. Chemother.,* 48, 267–273.

Compant S, Clément C, Sessitsch A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem*., 42:669–678.

Conti R, Chagas FO, Caraballo-Rodriguez AM, da Paixão Melo WG, do Nascimento AM et al. (2016). Endophytic actinobacteria from the Brazilian medicinal plant *Lychnophora ericoides* Mart. and the biological potential of their secondary metabolites. *Chem Biodivers*., 13:727–736.

Coombs JT, Franco CMM. (2003). Isolation and identification of actinobacteria from surface sterilized wheat roots. *Appl Environ Microbiol*., 69:5603e8.

Cushnie TT, Cushnie B, Lamb AJ. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int J Antimicrob Agents*, 44(5):377-386.

Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, et al. (2013). National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Intern. Med*., 173:1970–1978.

Dardeer HM, Taha AG, Elamary RB. (2020). Synthesis, Characterization and Antimicrobial Evaluation of Some Novel Anthracene Heterocycles Derivatives. *J Heterocycl Chemi*. 57:3951- 3960.

Datta R, Shah A, Huang SS, Cui E, Nguyen V, Welbourne SJ, et al. (2014). High nasal burden of methicillin-resistant *Staphylococcus aureus* increases risk of invasive disease. *J. Clin. Microbiol*., 52:312–314.

Davoodabadi F, Mobasherizadeh S, Mostafavizadeh K, Shojaei H, Havaei SA, Koushki AM, et al. (2016). Nasal colonization in children with community acquired methicillin-resistant *Staphylococcus aureus*. *Adv. Biom. Res*., 5:86.

De Bary A. (1866). Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten. Hofmeister's handbook of physiological Botany. W.Engelmann, Leipzig

de Kraker MEA, Davey PG, Grundmann H. (2011). Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteraemia: Estimating the burden of antimicrobial resistance in Europe. *PLoS Med*, 8:e1001104.

Debbab A, Aly AH, Edrada-Ebel R, Wray V, Pretsch A, Pescitelli G, Kurtan T, Proksch P. (2012). New Anthracene Derivatives-Structure Elucidation and Antimicrobial Activity. *Euro J Org Chem.*, 7:1351–1359.

Demain A. (1980). Microbial production of primary metabolites. Naturwissenschaften, 67(12):582-587.

Desai SS, Shruti K, Sana S, Sapna P, Zainab M, Swati H, Hungund BS. (2013). Phytochemical analysis, free radical scavenging and antioxidant profiling using chromatographic techniques for *Centella asiatica*. *Int J Biotechnol Bioeng Res*., 4(7): 687-696.

Devi KA, Pandey P, Sharma GD. (2016). Plant growth-promoting endophyte *Serratia marcescens* AL2-16 enhances the growth of *Achyranthes aspera* L., a medicinal plant. HAYATI J. Biosci. 23:173–180.

Devi S, Sharma M, Manhas RK. (2023). Purification and biological analysis of antimicrobial compound produced by an endophytic *Streptomyces* sp. *Scientific Reports*, 13(1), 15248.

Dini-Andreote F, Andreote FD, Araújo WL, Trevors JT, van Elsas JD. (2012). Bacterial genomes: habitat specificity and uncharted organisms. *Microb. Ecol.* 64:1–7.

Egamberdieva D, Wirth S, Behrendt U, Ahmad P, Berg G. (2017). Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. *Front microbiol.*, 8:199.

Ek-Ramos MJ, Gomez-Flores R, Orozco-Flores AA, Rodriguez-Padilla C, Gonzalez-Ochoa G, Tamez-Guerra P. (2019). Bioactive products from plant-endophytic gram-positive bacteria. *Front. Microbiol*., 10:463.

El Aila NA, Al Laham NA, Ayesh BM. (2017). Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at Al Shifa hospital in Gaza Strip. *BMC Infect. Dis*., 17:28.

El-Gendy MMAA, Mohamed ZK, Hekal NZ, Ali FM, Yousef AE. (2018). Production of bioactive metabolites from different marine endophytic *Streptomyces* species and testing them against methicillin-resistant *Staphylococcus aureus* (MRSA) and cancer cell lines. *BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology*, 99(1).

Elmagzob AAH, Ibrahim MM, Zhang GF. (2019). Seasonal diversity of endophytic bacteria associated with *Cinnamomum camphora* (L.) Presl. *Diversity*, 11:1–12.

Eloff JN. (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants. *J Ethnopharmacol*., 60:1–8.

Fiedler H, Bruntner C, Riedlinger J, Bull AT, Knutsen G, Goodfellow M et al. (2008). Proximicin A, B and C, novel aminofuran antibiotic and anticancer compounds isolated from marine strains of the actinomycetes Verrucosispora. *J Antibiot*., 61:158–163

Fouda A, Eid AM, Elsaied A, El-Belely EF, Barghoth MG, Azab E, et al. (2021). Plant growth-promoting endophytic bacterial community inhabiting the leaves of *Pulicaria incisa* (Lam.) DC inherent to arid regions. *Plants*, 10:76.

Fróes A, Macrae A, Rosa J, Franco M, Souza R, Soares R, Coelho R. (2012). Selection of a Streptomyces strain able to produce cell wall degrading enzymes and active against *Sclerotinia sclerotiorum*. *J Microbiol*, 50:798-806.

Gangwar M, Dogra S, Gupta UP, Kharwar RN. (2014). Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. *African. J. Microbiol. Res.* 8:184–191.

Gayathri P, Muralikrishnan V. (2013). Isolation and characterization of endophytic actinomycetes from mangrove plants for antimicrobial activity. *Int. J. Curr. Microbiol. Appl. Sci*., 2:78–79.

Ghasemzadeh A, Ghasemzadeh N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *J. Med. Plants Res*, 5(31): 6697-6703.

Gilbert GS, Diaz A, Bregoff HA. (2023). Seed disinfestation practices to control seed-borne fungi and bacteria in ho`me production of sprouts. *Foods*, 12(4), 747.

Gohil KJ, Patel JA, Gajjar AK. (2010). Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian J Pharm Sci* 72(5): 546.

Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M. (2015). Endophytic actinobacteria of medicinal plants: diversity and bioactivity. *Antonie Van Leeuwenhoek* 108:267–289.

González A, Vidal C, Espinoza D, Moenne A. (2021). Anthracene induces oxidative stress and activation of antioxidant and detoxification enzymes in *Ulva lactuca* (Chlorophyta). Sci Rep 11(1):7748.

Goodfellow M, Haynes JA. (1984). Actinomycetes in marine sediments. *Biological, biochemical and biomedical aspects of actinomycetes*, 453-472.

Gos F, Savi DC, Shaaban KA, Thorson JS, Aluizio R et al. (2017). Antibacterial activity of endophytic actinomycetes isolated from the medicinal plant *Vochysia divergens* (Pantanal, Brazil). *Front Microbiol.,* 8:1642.

Guimarães TC, Gomes TS, Fernandes CD, Barros FD, Oliveira KV, Bilal M, Bharagava RN, Ferreira LFR, Hollanda LM. (2020). Antitumor Microbial Products by Actinomycetes Isolated from Different Environments. *Role Microb. Communities Sustain.*, 113–160.

Hajji M, Jarraya R, Lassoued I, Masmoudi O, Damak M, Nasri M. (2010). GC/MS and LC/MS analysis, and antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis jalapa* tubers. Process Biochem., 45(9):1486–1493.

Hallmann J, Berg G, Schulz B .(2006). Isolation procedures for endophytic microorganisms. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, New York, 299–314.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. (1997). Bacterial endophytes in agricultural crops. *Can. J. Microbiol*. 43:895–914.

Hallmann J, Quadt-Hallmann A, Rodríguez-Kábana R, Kloepper JW. (1998). Interactions between Meloidogyne incognita and endophytic bacteria in cotton and cucumber. *Soil Biol. Biochem*., 30:925–937.

Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, de Lencastre H, Bentley SD, Kearns AM, Holden MT. (2017). Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. *Genome biology,* 18:1-1.

Hasanuzzaman M, Nahar K, Alam MM, Bhowmik PC, Hossain MA, Rahman MM, et al. (2014). Potential use of halophytes to remediate saline soils. *Biomed. Res. Int.*, 1–12.

Hayakawa M. (2008). Studies on the isolation and distribution of rare actinomycetes in soil. *Actinomycetologica,* 22(1):12-19.

Huang CB, Alimova Y, Myers TM, Ebersole, JL. (2011) Short-and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of oral biology*. 56(7):650-654.

Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T. et al. (2003). Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat. Biotechnol,*. 21:526.

Ippolito G, Leone S, Lauria FN, Nicastri E, Wenzel RP. (2010). Methicillin-resistant *Staphylococcus aureus*: the superbug. *Int J Infect Dis*, 14:S7-S11.

Jog R, Pandya M, Nareshkumar G, Rajkumar S. (2014). Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiology*, 160: 778–788.

Jørgensen PS, Wernli D, Folke C, Carroll SP. (2017). Changing antibiotic resistance: sustainability transformation to a pro-microbial planet. *Curr Opin Env Sust,* 25:66–76

Kabera JN, Semana, E, Mussa AR, He X. (2014). Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J. Pharm. Pharmacol*, 2(7):377-392.

Karthikeyan B, Joe MM, Islam MR, Sa T. (2012). ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. *Symbiosis*, 56:77–86.

Kato S, Shindo K, Yamagishi Y, Matsuoka M, Kawai H, Mochizuki, J. (1993). Phenazoviridin, a novel free radical scavenger from *Streptomyces* sp. Taxonomy, fermentation, isolation, structure elucidation and biological properties. *J. Antibiot*, 46:1485–1493.

Kaur DC, Chate SS. (2015). Study of antibiotic resistance pattern in methicillin resistant *Staphylococcus aureus* with special reference to newer antibiotic. *J. Glob. Infect. Dis*., 7:78.

Kawaguchi M, Minamisawa K. (2010). Plant-microbe communications for symbiosis. *Plant Cell Physiol*. 51:1377–1380.

Kenneth VIR. (2009). New antimicrobial agents for the treatment of bacterial infections in cancer patients. *Hem Onc*., 27:107-114.

Khirennas O, Mokrani S, Behira B, Bouras N, Driche EH, Moumen O. (2023). Isolation, Identification and Screening of Saharan Actinomycete

Strain *Streptomyces fimbriatus* AC31 Endowed with Antimicrobial Activity. *Int J Mol Cell Med*.12(1):51-69.

Kim MG, Jeong EY, Lee HS. (2009). Antimicrobial effects of anthracene and its derivatives against intestinal bacteria. *J Korean Soc Appl Biol Chem*, 52:327-330.

Kitahara T, Koyama N, Matsuda J, Aoyama Y, Hirakata Y, Kamihira S, Kohno S, Nakashima M, Sasaki H. (2004). Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant *Staphylococcus aureus*. *Biol Pharm Bull* ,27(9):1321-1326.

Kobayashi M, Kuzuyama T. (2020). Recent Advances in the Biosynthesis of Carbazoles Produced by Actinomycetes. *Biomolecules* , 10:1147.

Kohanski MA, Dwyer DJ, Collins JJ. (2010). How antibiotics kill bacteria: from targets to networks. *Nat Rev Microbiol*., 8(6):423-35.

Krause RM. (1992). The origin of plagues: old and new. *Science*, 257:1073–1078.

Krishnamurthy A, Nagel S, Orlov D. (2014). Sizing up repo. *J. Finance*, 69, 2381–2417.

Küçük HB, Yusufoğlu A, Mataracı E, Döşler S. (2011). Synthesis and biological activity of new 1, 3-dioxolanes as potential antibacterial and antifungal compounds. *Molecules*, 16(8): 6806-6815.

Kumar N, Singh RK, Mishra SK., Singh AK, Pachouri UC. (2010). Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. *Int J Microbiol Res*, 2(2):12.

Kurtboke DI. (2003). Selective isolation of rare actinomycetes. Nambour (Australia): Queensland Complete Printing Services.

Kuster E, Williams ST. (1964). Selection of media for isolation of streptomycetes. Nature (London) 202:928-929.

Kuster E. (1959). Outline of a comparative study of criteria used in characterization of the actinomycetes. *Int Bull Bacteriol Nomencl Taxon,* 9:97–104

Lalruatpuii E, Ngurtinkhuma RK, Lalruatfela S, Reddy KV. (2024). Usage and preservation of Mizo traditional medicine by the people of Chungtlang Village, Mamit District, Mizoram. *Journal of the Medical Library Association: JMLA*, 112(3):286.

Lalzarzovi ST, Lalramnghinglova H. (2016). Traditional use of medicinal plants found within Aizawl City in Mizoram, India. *Pleione* 10(2):269– 277.

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HFL, Sumpradit N, et al. (2013). Antibiotic resistance—the need for global solutions. *Lancet Infect. Dis*., 13:1057–1098.

Li Q, Zhu X, Xie Y, Liang J. (2021). Antifungal properties and mechanisms of three volatile aldehydes (octanal, nonanal and decanal) on *Aspergillus flavus*. *Grain & Oil Science and Technology*, 4(3), 131-140.

Liu K, Chen Q, Liu Y, Zhou X, Wang X. (2012). Isolation and biological activities of decanal, linalool, valencene, and octanal from sweet orange oil. J Food Sci 77(11):C1156-C1161.

Liu M, Abdel-Mageed WM, Ren B, He W, Huang P, Li X, Bolla K, Guo H, Chen C, Song F, Dai H. (2014). Endophytic *Streptomyces* sp. Y3111 from traditional Chinese medicine produced antitubercular pluramycins. *Applied microbiology and biotechnology*, 98:1077-1085.

Ludwig W, Klenk HP. (2005). Overview: a phylogenetic back bone and taxonomic framework for prokaryotic systemat ics. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn, vol 2, the Proteobacteria, part A, introductory essays. Springer, New York, 49–65

Ludwig-Müller J. (2015). Plants and endophytes: equal partners in secondary metabolite production?. *Biotechnology letters*, 37:1325-1334.

Mamatha A. (2011). Quantitative HPTLC Analysis of Andrographolide in Andrographis paniculata obtained from different geographical sources (India). *Int J Pharm Pharmaceut Sci.*, 3: 42-44.

Martens E, Demain AL. (2011). Platensimycin and platencin: promising antibiotics for future application in human medicine. *J Antibiot*. 64:705–710.

Matos D, Sá C, Cardoso P, Pires A, Rocha SM, Figueira E. (2019). The role of volatiles in Rhizobium tolerance to cadmium: effects of aldehydes and alcohols on growth and biochemical endpoints. *Ecotoxicol Environ Saf*., 186:109.

Matsukuma S, Okuda T, Watanabe J. (1994). Isolation of actinomycetes from pine litter layers. *Actinomycetologica*., 8(2):57–65.

Matsumoto A, Takahashi Y, Mochizuki M, Seino A, Iwai Y, Omura S. (1998). Characterization of actinomycetes isolated from fallen leaves. *Actinomycetologica*. 12(1):46–48.

Mediavilla JR, Patrawalla A, Chen L, et al. (2016). Colistin- and carbapenem-resistant *Escherichia coli* harboring mcr-1 and bla NDM-5, causing a complicated urinary tract infection in a patient from the United States: TABLE 1. *MBio* 7: e01191–e0111

Meenakshi S, Hiremath J, Meenakshi MH, Shivaveerakumar S. (2024). Actinomycetes: Isolation, Cultivation and its Active Biomolecules. *J Pure Appl Microbiol*.,18:1.

Mincer TJ, Jensen PR, Kauffman CA, Fenical W. (2002). Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl Environ Microbiol*, 68:5005–5011

Mishra VK, Passari AK, Chandra P, Leo VV, Kumar B, Gupta VK, Singh BP. (2017). Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from *Mirabilis jalapa* L. using UPLC-ESI-MS/MS and TD GC-MS. *PLoS One* 12(10):1–24.

Mitter B, Petric A, Shin MW, Chain PSG, Hauberg- Lotte L, Reinhold Hurek B, et al. (2013). Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Front. Plant Sci*., 4:120.

Miyadoh S. (1993). Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. Actinomycetologica, 9: 100-06.

Molyneux P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol.*,26(2):211-219.

Momin MD, Tripathi SK. (2018). Studies of endophytic actinomycetes associated with medicinal plants of Mizoram, Northeast, India. *Int J Curr Microbiol Appl Sci*, 7(12): 1398-1407.

Moyo SJ, Nkinda L, Majigo M, Mmbaga E, Mbembati N, Aboud S, et al. (2017). Prevalence of methicillin-resistant *Staphylococcus aureus* carriage on admission among patients attending regional hospitals in Dar es Salaam, Tanzania. *BMC Res. Notes*, 10:417.

Nafis A, Kasrati A, Azmani A, Ouhdouch Y, Hassani L. (2018). Endophytic actinobacteria of medicinal plant Aloe vera: Isolation, antimicrobial, antioxidant, cytotoxicity assays and taxonomic study. *Asian Pacific Journal of Tropical Biomedicine*, 8(10), 513-518.

Naine SJ, Devi CS, Mohanasrinivasan V. (2014). Antimicrobial, Antioxidant and Cytotoxic Activity of Marine *Streptomyces parvulus* VITJS11 Crude Extract. *Brazilian Archives of Biology and Technology*, 58:198-207.

Nair DN, Padmavathy S. (2014). Impact of endophytic microorganisms on plants, environment and humans. *Sci. World J*. 250693.

Namdar D, Charuvi D, Ajjampura V, Mazuz M, Ion A, Kamara I, et al. (2019). LED lighting affects the composition and biological activity of *Cannabis sativa* secondary metabolites. *Ind. Crop. Prod*. 132:177–185.

Nandhini SU, Sudha S, Jeslin VA, Manisha S. (2018). Isolation, identification and extraction of antimicrobial compounds produced by *Streptomyces* sps from terrestrial soil. *Biocatalysis and agricultural biotechnology*, 15:317-321.

Nelson RE, Jones M, Liu CF, Samore MH, Evans ME, Graves N, et al. (2015). The impact of healthcare-associated methicillin resistant *Staphylococcus aureus* infections on post-discharge healthcare costs and utilization. *Infect. Control Hosp. Epidemiol*., 36:534–542.

Okazaki T, Takahashi K, Kizuka M, Enokita R. (1995). Studies on actinomycetes isolated from plant leaves. Annual Report of Sankyo Research Laboratory 47:97-106.

Okunade AL. (2002). *Ageratum conyzoides* L. (Asteraceae). Fitoterapia. 73(1):1-16.

Okwori AEJ, Dina CO, Junaid S, Okeke IO, Adetunji JA, Olabode AO. (2007). Antibacterial activities of *Ageratum conyzoides* extracts on selected bacterial pathogens. *Internet J Microbiol*, 4(1), 34-56.

Oladunmoye MK. (2012). Antioxidant, free radical scavenging capacity and antimicrobial activities of *Mirabilis jalapa*. J Med Plants Res, 6(15):2909-2913.

O'Neill J. (2016). Tackling drug-resistant infections globally: final report and recommendations. 84

Orhan IE. (2012). *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evid Based Complement Alternat Med 1: 946259.

Ovsyannikova MN, Vol'Eva VB, Belostotskaya IS, Komissarova NL, Malkova AV, Kurkovskaya LN. (2013). Antibacterial activity of substituted 1, 3-dioxolanes. Pharm Chem J, 47:142-145.

Park JH, Choi JY, Son DJ, Park EK, Song MJ, Hellstrom M, Hong JT. (2017). Anti-inflammatory effect of titrated extract of *Centella asiatica* in phthalic anhydride induced allergic dermatitis animal model. Int J Mol Sci, 18(4):1–14.

Park KS. (2021). Pharmacological effects of *Centella asiatica* on skin diseases: Evidence and possible mechanisms. Evid Based Complement Alternat Med, 1: 5462633.

Passari AK, Leo VV, Singh G, Samanta L, Ram H, Siddaiah CN, Singh BP. (2020). In vivo studies of inoculated plants and in vitro studies utilizing methanolic extracts of endophytic *Streptomyces* sp. strain DBT34 obtained from *Mirabilis jalapa* L. exhibit ROS-scavenging and other bioactive properties. *International journal of molecular sciences*, 21(19), 7364.

Passari AK, Mishra VK, Saikia R, Gupta VK, Singh BP. (2015). Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. *Frontiers in microbiology*, 6: 273.

Passari AK, Mishra VK, Singh G, Singh P, Kumar B, Gupta VK, Singh BP. (2017). Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. *Scientific Reports*, 7(1), 11809.

Pathare NA, Asogan H, Tejani S, Al Mahruqi G, Al Fakhri S, Zafarulla R., et al. (2016). Prevalence of methicillin resistant *Staphylococcus aureus* [MRSA] colonization or carriage among health-care workers. *J. Infect. Public Health*, 9:571–576.

Petrini O, Sieber TN, Toti L,Viret O. (1992). Ecology, metabolite production, and substrate utilization in endophytic fungi. *Nat Tox*, 1:185–196

Petrini O. (1991). ”Fungal endophytes of tree leaves,” in Microbial Ecology of Leaves, eds J. H. Andrews and S. S. Hirano (New York, NY: Springer-Verlag), 179–197.

Phuakjaiphaeo C, Kunasakdakul K. (2015). Isolation and screening for inhibitory activity on *Alternaria brassicicola* of endophytic actinomycetes from *Centella asiatica* (L.) Urban. *J Agri Technol*, 11(4):903-912

Pimentel MR, Molina G, Dionisio AP, Maróstica MR, Pastore GM. (2011). Use of endophytes to obtain bioactive compounds and their application in biotransformation process. *Biotechnol. Res. Int*. 2011:576286.

Pittella F, Dutra RC, Junior DD, Lopes MT, Barbosa NR. (2009). Antioxidant and cytotoxic activities of *Centella asiatica* (L) Urb. *Int J Mol Sci*, 10(9):3713–21.

Platzer M, Kiese S, Herfellner T, Schweiggert-Weisz U, Miesbauer O, Eisner P. (2021). Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. *Molecules*, 26(5):1244.

Popiołek Ł. (2021). Updated information on antimicrobial activity of hydrazide–hydrazones. *Int J Mol Sci*, 22(17): 9389.

Popiołek, Ł. (2017). Hydrazide–hydrazones as potential antimicrobial agents: overview of the literature since 2010. *Medicinal Chemistry Research*, 26:287-301.

Poschenrieder C, Fernández JA, Rubio L, Pérez L, Terés J, Barceló J. (2018). Transport and use of bicarbonate in plants: current knowledge and challenges ahead. *Int J Mol Sci*, 19(5):1352.

Praptiwi, Fathoni A, Putri AL, Wulansari D, Agusta A. (2019). Assessment of actinomycetes isolated from soils on Simeuleu Island as antibacterial and antioxidant. In *AIP Conference Proceedings*, 2120,(1):080011). AIP Publishing LLC.

Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, et al. (2009). Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl. Environ. Microbiol*. 75:6176–6186.

Quach NT. (2020). Functional Analyses of Thiol Switches and Their Impact on the Mycothiol Redox Potential in Actinomycetes. Ph.D. Thesis, Freie University of Berlin, Berlin, Germany.

Rammali S, Kamal FZ, El Aalaoui M, Rahim A, Baidani A, Dari K, Bencharki B. (2024). Exploring the Antimicrobial and Antioxidant Activities of *Streptomyces* sp. EIZ2 Isolated from Moroccan Agricultural Soil. *Microbiology Research*, 15(2):762-786.

Raskildina GZ, Ishmetova DV, Sokov SA, Golovanov AA. (2024). Cytotoxic and Antioxidant Activity of a Series of 4-Methylene-1, 3-Dioxolane Acetals. Pharm Chem J, 58(4):631-633.

Ravichandiran P, Sheet S, Premnath D, Kim AR, Yoo DJ. (2019). 1, 4-Naphthoquinone analogues: Potent antibacterial agents and mode of action evaluation. *Molecules*, 24(7): 1437.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*, 26(9-10): 1231–1237.

Rosenblueth M. Martínez-Romero E. (2006). Bacterial endophytes and their interactions with hosts. *Mol. Plant Microbe Interact*. 19:827–837.

Rostaminejad B, Karimi AR, Dinari M, Hadizadeh M. (2023). Photosensitive chitosan-based injectable hydrogel chemically cross-linked by perylene bisimide dopamine with robust antioxidant and cytotoxicity enhancer properties for in vitro photodynamic therapy of breast cancer. *ACS Applied Bio Materials*, 6(3):1242-1251.

Rostaminejad B, Karimi AR, Dinari M, Hadizadeh M. (2024). Emergence of in vitro antimicrobial photodynamic activities of perylene-dopamine and enhancement of its phototoxicity properties via chelating with FeIII and encapsulating in CMC-based safe hydrogel film. *J Photochem Photobiol A: Chem*, 449:115358.

Rüngeler P, Lyss G, Castro V, Mora G, Pahl HL, Merfort I. (1998). Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF-kappa B and enzymes of the arachidonic acid pathway as targets. *Planta Med*, 64(7):588-93.

Rustamova N, Wubulikasimu A, Mukhamedov N, Gao Y, Egamberdieva D, Yili A. (2020). Endophytic bacteria associated with medicinal plant *Vernonia anthelmintica*: diversity and characterization. *Curr. Microbiol*, 77:1457–1465.

Rutala WA, Weber DJ. (2021). Disinfection and sterilization in health care facilities: an overview and current issues. *Infectious Disease Clinics*, 35(3):575-607.

Saadoun I, Muhana A. (2008). Optimal production conditions, extraction, partial purifcation and characterization of inhibitory compound(s) produced by *Streptomyces* Ds-104 isolate against multi-drug resistant *Candida albicans*. *Curr Trends Biotechnol Pharm*, 2:402–20.

Sahu PK, Tilgam J, Mishra S, Hamid S, Gupta A K J, Verma SK, Kharwar RN. (2022). Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *Journal of Basic Microbiology*, 62(6):647-668.

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4:406-425.

Schultes RE. (1960). Tapping our heritage of ethnobotanical lore. Econ Bot. 14:257-262.

Schulz B, Boyle C. (2006). Microbial root endophytes. In: Sieber TN (ed) What are endophytes? *Springer, Berlin*, 9:1–13

Schulz B, Wanke U, Draeger S, Aust HJ. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological research*, 97(12):1447-1450.

Schulz D, Nachtigall J, Riedlinger J, Schneider K, Poralla K, Imhoff JF et al. (2009). Piceamycin and its N-acetylcysteine adduct is produced by *Streptomyces* sp. GB 4-2. *J Antibiot*, 62:513–518

Shabira AP, Tjahjoleksono A, Lestari Y. (2022). Endophytic actinobacteria of Eleutherine palmifolia as antioxidant producer. *Biodiversitas Journal of Biological Diversity*, 23(8).

Shaikh AF, Elfeki M, Landolfa S, Tanouye U, Green SJ, Murphy BT. (2015). Deuteromethylactin B from a Freshwater-derived *Streptomyces* sp. *Natural Product Sciences*, 21(4), 261-267.

Shankar R, Rawat M. (2012). Conservation of traditional medicinal practices and pharmaceutically important medicinal plants in Mizoram, India. *Journal of Biology and Life Science*, 4(2):95â.

Sharma HK, Chhangte L, Dolui AK. (2001). Traditional medicinal plants in Mizoram, India. *Fitoterapia*, 72:146–161.

Sharma M. (2014). Actinomycetes: source, identification, and their applications. *Int J Curr Microbiol Appl Sci*, 3(2):801–832

Shekar P, Kumar KS, Gopikrishnan V, Radhakrishnan M. (2016). HPTLC fingerprint profile of antibacterial compound produced from forest soil Streptomyces SFA5. *Bangladesh Journal of Pharmacology*, 11(2):295-300.

Shen T, Chen L, Liu Y, Shi S, Liu Z, Cai K, Chengshui L, Wang C. (2021). Decanoic acid modification enhances the antibacterial activity of PMAP-23RI-Dec. *European Journal of Pharmaceutical Sciences*, 157:105609.

Sheng HM, Gao HS, Xue LG, Ding S, Song CL, Feng HY, et al. (2011). Analysis of the composition and characteristics of culturable endophytic bacteria within subnival plants of the Tianshan Mountains, Northwestern China. *Curr. Microbiol*, 62:923–932.

Shindo K, Misawa N. (2014). New and Rare Carotenoids Isolated from Marine Bacteria and Their Antioxidant Activities. *Mar. Drugs*, 12:1690–1698.

Shirling EB and Gottlieb D. (1966). Methods for characterization of *Streptomyces* species. *Int. J.Syst.Bacteriol*, 16:313–340

Shomurat T, Yoshida J, Amano S, Kojina M, Niida T. (1979). Studies on actinomycetal producing antibiotics only in agar culture. I. Screening taxonomy and morphology – productivity relationship of *Streptomyces halstedii*, strain SF. *J Antibiot*, 32:427-435.

Shoun H, Kano M, Baba I, Takaya N and Matsuo M. (1998). Dentrification by actinomycetes and purification of dissimilatory nitrite reductase and azurin from *Streptomyces thioluteus*. *J Bacteriol*., 180: 4413-4415

Singh G, Passari AK, Singh BP, Senthil N. (2017). Traditionally Used Medicinal Plants Belongs to Family Asteraceae for the Treatment of Cancer in Mizoram, Northeast India.

Singh M, Kumar V, Singh I, Gauttam V, Kalia A. (2010). Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa* Linn. leaves. *Pharmacogn Res* 2(6):364.

Singh R, Dubey AK. (2015). Endophytic actinomycetes as emerging source for therapeutic compounds. *Indo Global, J. Pharm. Sci*. 5: 106–116.

Singh S, Kumar P, Gopalan N, Shrivastava B, Kuhad R, Chaudhary HS. (2012). Isolation and partial characterization of actinomycetes with antimicrobial activity against multidrug resistant bacteria. *Asian Pac J Trop Biomed,* 2(2): S1147–S1150.

Singleton VL, Rossi JA. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* , 16(3):144-158.

Sivasankaridevi T, Rajan A, Maina CC, Suvarna VC. (2013). Antimicrobial activity of some important edible leaf extracts. *Insight Microbiology*, 3(2):15-18.

Smee DF, Huffman JH, Morrison AC, Barnard DL, Sidwell RW. (2001). Cyclopentane neuraminidase inhibitors with potent in vitro anti-influenza virus activities. *Antimicrob Agents Chemother.,* 45(3):743-8.

Song X, Wu H, Yin Z, Lian M, Yin C. (2017). Endophytic bacteria isolated from Panax ginseng improves ginsenoside accumulation in adventitious Ginseng root culture. *Molecules*, 22:837–849.

Soren AD, Lalthanpuii PB. (2021). A traditional and pharmacological approach of medicinal plants in Mizoram, India. In *Natural Drugs from Plants*. IntechOpen.

Strobel G, Daisy B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and molecular biology reviews*, 67(4):491-502.

Strobel GA. (1998). Long DM. Endophytic microbes embody pharmaceutical potential. *ASM News,* 64:263e8.

Subbulakshmi GK, Thalavaipandian A, Bagyalakshmi RV, Rajendran A. (2012). Bioactive endophytic fungal isolates of *Biota orientalis* (L) Endl, *Pinus excelsa* wall and *Thuja occidentalis* L. *Int. J. Adv. Life Sci*. 4:9–15.

Suzuki N, Ohtaguro N, Yoshida Y, Hirai M, Matsuo H, Yamada Y, et al. (2015). A compound inhibits biofilm formation of *Staphylococcus aureus* from Streptomyces. *Biol. Pharm. Bull.*, 38:889–892.

Taechowisan T, Lumyong S. (2003). Activity of endophytic actinomycetes from roots of *Zingiber officinale* and *Alpinia galena* against phytopathogenic fungi. *Ann Microbiol*, 53:291–298.

Tailliez P, Pagès S, Ginibre N, Boemare N. (2006). New insight into diversity in the genus Xenorhabdus, including the description of ten novel species. Int J Syst Evol Microbiol, 56(Pt 12):2805-2818

Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10:512-526.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38(7):3022-3027.

Tan LTH; Chan KG, Pusparajah P, Yin WF, Khan TM, Lee LH, Goh BH. (2019). Mangrove derived *Streptomyces* sp. MUM265 as a potential source of antioxidant and anticolon-cancer agents. *BMC Microbiol.*, 19:1–16.

Tan RX, Zou WX. (2001). Endophytics: a rich source of functional metabolites. *Nat Prod Rep*,18:448-59.

Tan SC, Bhattamisra SK, Chellappan DK, Candasamy M. (2021). Actions and therapeutic potential of madecassoside and other major constituents of *Centella asiatica*: a review. *Appl Sci*, 11(18):8475.

Tangjitjaroenkun J, Pluempanupat W, Tangchitcharoenkhul R, Yahayo W, Supabphol R. (2021). Antibacterial, antioxidant, cytotoxic effects and GC-MS analysis of mangrove-derived *Streptomyces achromogenes* TCH4 extract. *Archives of Biological Sciences*, 73(2):223-235.

Tikhomirov AS, Sinkevich YB, Dezhenkova LG, Kaluzhny DN, Ilyinsky NS, Borshchevskiy VI, Schols D, Shchekotikhin AE. (2024). Synthesis and antitumor activity of cyclopentane-fused anthraquinone derivatives. *Eur J Med Chem*., 5;265:116103.

Tiwari R, Kalra A, Darokar MP, Chandra M, Aggarwal N, Singh AK, et al. (2010). Endophytic bacteria from *Ocimum sanctum* and their yield enhancing capabilities. *Curr. Microbiol*. 60:167–171.

Tiwari R, Rana CS. (2015). Plant secondary metabolites: a review. *International Journal of Engineering Research and General Science*, 3(5):661-670.

Tona L, Kambu K, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totté J, Vlietinck AJ. (1999). Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*. 6(1):59-66.

Torbati FA, Ramezani M, Dehghan R, Amiri MS, Moghadam AT, Shakour N, Elyasi S, Sahebkar A, Emami SA. (2021). Ethnobotany, phytochemistry and pharmacological features of *Centella asiatica*: a comprehensive review. *Adv Exp Med Biol*, 1308:451–99.

Tran TT, Munita JM, Arias CA. (2015). Mechanisms of drug resistance: daptomycin resistance. *Ann N Y Acad Sci*.1354:32-53.

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, 5(3):93.

Uematsu H, Yamashita K, Kunisawa S, Fushimi K, Imanaka Y. (2017). Estimating the disease burden of methicillin-resistant *Staphylococcus aureus* in Japan: retrospective database study of Japanese hospitals. *PLoS ONE*, 12:e0179767.

Uzma F, Hashem A, Murthy N, Mohan HD, Kamath PV, Singh BP, Venkataramana M, Gupta VK, Siddaiah CN, Chowdappa S, Alqaeawi AA, Abd-Allah EF. (2018). Endophytic fungi— alternative sources of cytotoxic compounds: a review. *Front Pharmacol*, 9(309):1–37.

Vendan RT, Yu YJ, Lee SH. et al. (2010). Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. *J Microbiol,*. 48:559–565

Villano D, Fernandez-Pachon MS, Moya ML, Troncoso AM, Garcıa-Parrilla MC. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radicals. *Talanta,*. 71:230–235

Waheeda K, Shyam KV. (2017). Formulation of novel surface sterilization method and culture media for the isolation of endophytic actinomycetes from medicinal plants and its antibacterial activity. *J Plant Pathol Microbiol*, 8(399): 2.

Wainwright M. (1991). Streptomycin: discovery and resultant controversy. *Hist Philos Life Sci*, 13:97–124

Wang J, Soisson SM, Young K, Shoop W, Kodali S et al. (2006). Platensimycin is a selective FabF inhibitor with potent antibiotic properties. *Nature*, 441:358–361.

Watve M, Tickoo R, Jog M, Bhole B. (2001). How many antibiotics are produced by the genus Streptomyces ?. *Arch Microbiol*, 176(5):386–390.

Wilson D. (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos*, 73:274–276.

Wippel K. (2023). Plant and microbial features governing an endophytic lifestyle. *Curr Opin Plant Biol*, 76:102483.

Wood WF, Szewczak JM. (2007). Volatile antimicrobial compounds in the pelage of the Mexican free-tailed bat, *Tadarida brasiliensis* mexicana. *Biochem Syst Ecol*, 35(9):566-568.

Wu Q, Er-bu A, Liang X, Luan S, Wang Y, Yin Z, Song X. (2020). Evaluation of antioxidant and anticancer activities of naphthoquinones-enriched ethanol extracts from the roots of *Onosma hookeri* Clarke. var. longiforum Duthie. *Food Sci Nutr*, 8(8): 4320-4329.

Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, Van der Lelie D. (2010). Comparative genomics and functional analysis of niche specific adaptation in *Pseudomonas putida*. *FEMS Microbiol Rev*, 35(2):299–323.

Yang D, Teng Q, Dong LB, CrnovcIc I, Huang T, Ge H, et al. (2017). Genome mining of *Streptomyces mobaraensis* DSM40847 as a bleomycin producer providing a biotechnology platform to engineer designer bleomycin analogues. *Org. Lett.* 19:1386–1389.

Yang MH, Lee M, Deivasigamani A, Le DD, Mohan CD, Hui KM Sethi G, Ahn KS. (2023). Decanoic acid exerts its Anti-tumor effects via Targeting c-Met Signaling cascades in Hepatocellular Carcinoma Model. *Cancers*, 15(19):4681.

Yang X, Yang Y, Peng T, Yang F, Zhou H et al. (2013). A new cyclopeptide from endophytic *Streptomyces* sp. YIM 64018. *Nat Prod Commun*, 8:1753–1754.

Yılmaz C, Güner P, Aşkun T, Yükrük F. (2023). Novel Perylene-Based Antimicrobial PDI Chromophores. *J Turk Chem Soc Sec A: Chem*, 10(3):633-640.

Yin J, Kwon GJ, Wang MH. (2007). The antioxidant and cytotoxic activities of *Sonchus oleraceus* L extracts. *Nutr Res Pract*, 1(3):189–194.

Zachariah SM, Aleykutty NA, Viswanad V, Jacob S, Prabhakar V. (2011). In-vitro Antioxidant Potential of Methanolic Extracts of *Mirabilis jalapa* Linn. *Free Radicals and Antioxidants*, 1(4):82–86.

Zeb A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *J. Food Biochem.* 44.

Zhang Q, Acuna JJ, Inostroza NG, Mora ML, Radic S, Sadowsky MJ, et al. (2019). Endophytic bacterial communities associated with roots and leaves of plants growing in Chilean extreme environments. *Sci. Rep.* 9:4950–4962.

Zhang X, Ren K, Zhang L. (2012). Screening and preliminary identification of medicinal plants endophytic actinomycetes used for inhibiting penicillin-resistant *Staphylococcus aureus. Int J Biol,* 4(2):119–124

Zhao K, Penttinen P, Guan T, Xiao J, Chen Q, Xu J, et al. (2011). The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in Panxi Plateau China. *Curr. Microbiol*, 62:182–190.

Zhishen J, Mengcheng T, Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, 64(4): 555-559.

Zin NM, Sarmin NI, Ghadin N, Basri DF, Sidik NM, Hess WM, Strobel GA. (2007). Bioactive endophytic Streptomycetes from the Malay Peninsula. *FEMS Microbiol Lett* 274:83-88.

BIODATA

Name	: LALROKIMI
Gender	: FEMALE
Organization	: MIZORAM UNIVERSITY
Address	: MISSION VENGTHLANG, AIZAWL, MIZORAM
Phone	: 8939089954
Email	: lalrokimitochhawng@gmail.com

Educational Background

Qualification	Board/University	Year	Division	%	CGPA
M.Phil	Mizoram University	2019	Distinction	-	9.0
M.Sc	University of Madras	2017	Distinction	-	8.0
B.Sc	University of Madras	2015	Distinction	-	8.1
Class XII	MBSE	2012	First	71.4	-
Class X	MBSE	2009	First	67.2	-

PUBLICATIONS

1. **Lalrokimi,** Deka P, Carrie W, Lallawmsangi, Sailo CV, Lalrosangpuii, Lalremruati F, Fanai A, Umbon Y, Lalnunmawii E, Zothanpuia (2025). Antibacterial potential and chromatographic profiling of bioactive compounds from endophytic *Streptomyces* sp. strain MIRK71 isolated from *Mirabilis jalapa* (L.) *PeerJ* 13:e19683.
2. **Lalrokimi**, Fanai, A., Lalremruati, F., Bohia, B., Lalhriatpuii, N., Lalrosangpuii, ... & Lalnunmawii, E. (2025). *Streptomyces* sp. strain CENK12 from *Centella Asiatica* L.: a potential source of antimicrobial and antioxidant agents against multidrug-resistant *Staphylococcus aureus*. *Molecular Biology Reports*, *52*(1), 845.
3. Zorem, L., Bohia, B., Lalmuanpuii, R., Lalremruati, F., Fanai, A., Lalhriatpuii, N., **Lalrokimi**…& Singh, P. K. (2026). Ethnobotanical validation and phytochemical profiling of *Glinus oppositifolius* (L.) Aug. DC used by indigenous tribes of Mizoram. *Discover Food*.
4. **Lalrokimi,** Mehetre G, Zothanpuia, Singh BP, Yadav MK, Lalnunmawii E. (2023). Cyanobacteria-mediated heavy metal and xenobiotic bioremediation. In *Cyanobacterial Biotechnology in the 21st Century*. 275-283. Singapore: Springer Nature Singapore.
5. **Lalrokimi,** Malvi Y, Singh BP, Zothanpuia. (2022). Antimicrobial Resistance in Environmental Microbiome: An Overview. *Understanding the Microbiome Interactions in Agriculture and the Environment*, 11-22.
6. Lalrosangpuii, **Lalrokimi**. (2021). A review on the phytochemical properties of five selected genera of orchids. *Sci Vision.* 2: 50-58.
7. **Lalrokimi T**, Mishra VK, Passari AK, Singh BP. (2019). Endophytic fungi: role in dye decolorization. *Advances in endophytic fungal research: present status and future challenges.* 1-15.
8. Mehetre GT, Deka P, Carrie W, **Lalrokimi**, Singh BP. (2022). Thermophilic and thermotolerant cyanobacteria: environmental and biotechnological perspectives. *Cyanobacterial lifestyle and its applications in biotechnology*. eds. P. K. Singh and M. F. Fillat (Amsterdam: Elsevier Inc.). 159–178.
9. Fanai A, Bohia B, Lalremruat, F, Lalhriatpuii N, **Tochhawng L**, Lalmuanpuii

R, Singh PK. (2025). Fusarium spp. induce diseases in ginger: nature of pathogen, pathogenesis and management. *Microbial Pathogenesis*, 107597.

10. Bohia B, Fanai A, Lalmuanpuii R, Zothanpuia, Lalremruati F, Lalhriatpuii N, **Lalrokimi**, Singh PK, Yadav MK. (2024). Traditional Ethnomedicinal Plants: A Focus on the Tribals of Mizoram, India. In *Ethnomedicinal Plants for Drug Discovery: Current Developments*, 125-160. Singapore: Springer Nature Singapore.
11. Fanai A, Bohia B, Lalremruati F, Lalhriatpuii N, **Lalrokimi**, Lalmuanpuii R, Singh PK, Zothanpuia. (2024). Plant growth promoting bacteria (PGPB)-induced plant adaptations to stresses: an updated review. PeerJ.20;12:e17882.
12. Lalremruati, F., Lalhriatpuii, N., Fanai, A., Bohia, B., **Lalrokimi**, Lalmuanpuii, R., ... & Zothanpuia. (2024). Fungal endophytes as biocontrol agents of plant pathogens: recent developments and prospects. *Endophytic Fungi: The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries.* 279-294.

PRESENTATION

1. **Lalrokimi**, Zothanpuia., Esther Lalnunmawii (2023). Isolation and Identification of Endophytic Actinobacteria from Medicinal Plants as a potential source against clinical MDR pathogens. National conference on Biotechnology for Sustainable Agriculture and Human Health Organized by the Department of Biotechnology, Pachhunga University College (PUC), Aizawl, India
2. **Lalrokimi**, Zothanpuia, Esther Lalnunmawii (2023). In PUC Research Conclave 2023 (An International Workshop on Research and Project Development) Organized by Research and Development Cell, Pachhunga University College, Aizawl, India
3. **Lalrokimi**, Lallawmsangi, Zothanpuia, Esther Lalnunmawii. (2023). Endophytic Actinobacteria Associated with Medicinal Plants as a potential source against clinical MDR pathogens. National Seminar on Emerging Trends in Biological Sciences: A North East India Perspective organized by the

Department of Biotechnology and Bioinformatics, North-Eastern Hill University (NEHU), Shillong, India

4. **Lalrokimi**, Imtisunep Longchar, Esther Lalnunmawii. (2023). Evaluation of Antimicrobial, Antioxidant, Phytochemical and Cytotoxic potential of Sacha Inchi (*Plukenetia volubilis*). National seminar on Biotechnology for Sustainable Biosphere organised by the Department of Biotechnology, Mizoram University, Aizawl, India.

WORKSHOP/SEMINARS ATTENDED

1. National Seminar on Biotechnology for Sustainable Biosphere organized by the Department of Biotechnology, Mizoram University, Mizoram on the 30th June and 1st July 2023
2. National Seminar on Emerging Trends in Biological Sciences: A North East India Perspective organized by the Department of Biotechnology and Bioinformatics, North-Eastern Hill University (NEHU), Shillong, India from 28th February to 1st March, 2023
3. PUC Research Conclave 2023 (An International Workshop on Research and Project Development) Organized by Research and Development Cell, Pachhunga University College, Aizawl, India from 24th – 25th January, 2023
4. National conference on Biotechnology for Sustainable Agriculture and Human Health Organized by the Department of Biotechnology, Pachhunga University College (PUC), Aizawl, India from 12-13 June, 2023
5. Two Days Skill Development Program on Green Farming organized by Department of Biotechnology, Mizoram University & Department of Horticulture, Aromatic & Medicinal Plants (HAMP), Mizoram University and ICAR-NBAIM, Uttar Pradesh on 27th-28th February, 2020
6. Two days Skill Development Program on Organic Farming For Sustainable development of NE Farmers organized by the Departhment of Biotechnology, Mizoram University, Pachhunga University College Campus, Mizoram from 24th to 25th March, 2021
7. Training on Analysis of Bioactive compounds using High- Performance Liquid

Chromatography (HPLC) held on 10th to 15th October, 2020 organised by the MZU BioNEST-BEM Incubation Centre, Mizoram University, Mizoram

8. Three days Skill Development Programme on mushroom cultivation for NER organized jointly by Rajiv Gandhi national Institure of Youth Development under its Youtj Led Sustainable Development Programme (YLSDP) IN Higher Educational Instutions, Mizoram from 10th -12th February, 2021

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE: Lalrokimi

DEGREE: Ph.D.

DEPARTMENT: Biotechnology

TITLE OF THE THESIS: Antimicrobial potential of endophytic actinobacteria associated with selected traditional medicinal plants against clinical MDR pathogens

DATE OF ADMISSION: 26/07/2019

APPROVAL OF RESEARCH PROPOSAL

1. DRC: 26/05/2020

2. BOS: 29/05/2020

3. SCHOOL BOARD: 12/6/2020

MZU REGISTRATION NO.: 1703966

Ph.D. REGISTRATION NO. & DATE: MZU/Ph. D./1425 of 26.07.2019

EXTENSION (IF ANY): 2 years (N0.16-2/MZU(Acad)/24/202-205
Dated the 22nd July,2025)

Head

Department of Biotechnology

ABSTRACT

ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC ACTINOBACTERIA ASSOCIATED WITH TRADITIONAL MEDICINAL PLANTS AGAINST MDR PATHOGENS

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

LALROKIMI

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INTRODUCTION

Natural products, particularly those obtained from the vast variety of plants and microorganisms, remain an essential source for discovering new and potentially bioactive compounds that can be utilized in drug development. Traditional medicinal plants have garnered significant interest from researchers due to their therapeutic effects on various health concerns. Furthermore, these plants contain an interesting assortment of endophytic actinobacteria, which are filamentous, gram-positive bacteria that inhabit the plant tissues. (Golinska et al., 2015). These endophytes play a crucial role in maintaining the ecological equilibrium of their host plants and could also contain distinctive chemical compounds with valuable medicinal attributes, thereby boosting the prospects of traditional medicine within contemporary health care (Tiwari et al., 2023). Endophytic actinobacteria represent a remarkable source of bioactive natural compounds discovered in higher plants that thrive in a variety of environments, although they remain a relatively overlooked category of microorganisms. These organisms inhabit the internal tissues of plants, usually drawing nutrients and shelter from their host. In return, they improve the host's overall fitness by generating a range of bioactive metabolites and providing protection to the plant (Strobel and Daisy, 2003). The persistent rise of infections resistant to antibiotics remains a significant issue for healthcare worldwide (Spellberg et al., 2008). At present, methicillin-resistant Staphylococcus aureus (MRSA) is the most commonly identified antibiotic-resistant pathogen in various areas worldwide, including Europe, the Americas, North Africa, the Middle East, and East Asia (Grundmann 2006). The genus Streptomyces, a type of actinobacteria, has been extensively acknowledged as a prolific source of valuable secondary metabolites. It remains a significant contributor to the ongoing development of new bioactive compounds, underscoring its importance in both biotechnological applications and pharmaceutical research. (Berdy 2012). Many marketed antibiotics are derived from this genus (Pelaez 2006). The secondary metabolites generated by actinomycetes demonstrate a diverse array of biological activities, such as antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties (Selim et al., 2021).

During normal cellular metabolism, both animals and plants generate reactive oxygen species (ROS), which are highly reactive molecules that can cause damage to cellular structures. This condition is harmful, as it can result in oxidative DNA damage, a process that has been linked to the onset of a wide array of health issues. These issues include serious conditions such as cardiovascular diseases, atherosclerosis (the hardening of arteries), reperfusion injury (tissue damage caused when blood supply returns after a period of ischemia), and cataract formation, along with chronic ailments like rheumatoid arthritis and various inflammatory disorders. Moreover, oxidative stress has also been implicated in the development of cancer (Reuter et al., 2010). To combat oxidative damage, many synthetic antioxidants have been developed, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG). These compounds are designed to slow down the oxidation process and help protect cells from ROS. However, their usage comes with significant caution, as they must be strictly regulated due to potential health hazards associated with their prolonged consumption (Xu et al., 2021). The crucial contribution of free oxygen radicals to the onset and advancement of various diseases has motivated researchers to investigate new and effective antioxidant substances sourced from microorganisms. These substances may act as significant therapeutic agents. Through the examination of a variety of microbial origins, scientists strive to discover antioxidants that can counteract damaging free radicals, thus providing innovative solutions for conditions associated with oxidative stress, including cancer, heart diseases, and neurodegenerative illnesses. This research not only aims to deepen our comprehension of microbial metabolism but also highlights the significance of utilizing nature's resources for health advantages.

The present study focuses on isolating actinobacteria from the tissues of the medicinal plants of Mizoram, India. It also aims to evaluate the antimicrobial and antioxidant activities of the secondary metabolites produced by these actinobacteria, as well as identify the bioactive compounds present in the methanol extract of the actinobacteria.

OBJECTIVES

- Isolation and identification of endophytic actinobacteria from selected medicinal plants of Mizoram forests.
- Antimicrobial screening of endophytic actinobacteria against clinical multi-drung resistance (MDR) clinical bacterial pathogens.
- Extract preparation, purification and characterization of antimicrobial compounds from selected potential isolates.
- Validation of purified bioactive compounds for their antimicrobial potency against MDR clinical pathogens.

MATERIALS AND METHODS

Selection and collection of medicinal plants

Five traditional medicinal plants from Mizoram were selected based on a literature review: *Mikania micrantha*, *Centella asiatica* (L.) Urb., *Tithonia diversifolia*, *Mirabilis jalapa* L., and *Clerodendrum colebrookianum* Walp. The various parts of the plants were collected in sterile containers and brought back to the laboratory. They were thoroughly washed with tap water and then dried at room temperature overnight.

Isolation of Endophytic Actinobacteria

Endophytic actinobacteria were isolated from five selected medicinal plants. Tissues from the roots, leaves, and stems of these plants were used for the isolation of actinobacteria. After surface sterilization (Taechowisan and Lumyong, 2003) the tissues were kept in five different nutritional media to obtain maximum diversity i.e. starch casein agar (SCA), actinomycetes isolation agar (AIA), tyrosine agar medium (ISP7), glycerol asparagine agar base (ISP5), and King's medium containing nalidixic acid and cycloheximide (50 μg/mL) to suppress the growth of Gram-negative bacteria and fungi, respectively. The dried tissues were kept in the media, and the plates were incubated at 28±2°C in a BOD incubator. Microbial growth was observed, and actinobacteria were morphologically selected (Shirling and Gottlieb, 1966; Goodfellow and Haynes, 1984).

Collection of Clinical MDR pathogens

Clinical pathogens were ccollected from patients at Synod Hospital, in Durtlang, Aizawl, Mizoram. To ensure patient confidentiality, a numbering system was employed to label the collected pathogens. Each clinical isolate underwent a thorough process of identification using the Vitek®MS system (bioMérieux, Marcy l'Etoile, France), specifically utilizing the GP colorimetric identification card to determine their precise nature. Additionally, the antibiotic susceptibility of these pathogens was assessed through the Vitek®MS AST system, again from bioMérieux. A comprehensive list of the clinical pathogens identified during this study is presented in Table 1.

Table 1: Test organisms (MDR pathogens) used for the antimicrobial screening

S.No	Pathogens	Organisms	Resistance
1.	17	*Escherichia coli*	Meropenem, Ciprofloxacin, Trimethoprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem
2.	31	*Escherichia coli*	Meropenem,Amikacin, Gentamicin, Ciprofloxacin,Trimethprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem
3.	038	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
4.	161	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

5.	363	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
6.	365	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
7.	452	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
8.	536	*Enterococcus faecalis*	Vancomycin, Benzylpenicillin, Gentamicin, Daptomycin, Teicoplanin, Tetracycline, Ciprofloxacin, Erythromycin, Levofloxacin
9.	622	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
10.	645	*Staphylococcus aureus*	Rifampicin, Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
11.	721	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Erythromycin,
12.	790	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

13.	809	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
14.	880	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
15.	924	*Staphylococcus aureus*	Rifampicin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
16.	995	*Klebsiella pneumoniae*	Meropenem, Gentamicin, Ciprofolxacin, Tigecycline, Trimethoprim, Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem,
17.	1097	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Vancomycin, Tetracyclin, Nitrofurantoin, Benzylpenicillin, Gentamicin, Ciprofloxacin, Erythromycin, Levofloxacin
18.	1098	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
19.	1219	*Enterococcus faecalis*	Vancomycin, Tetracycline, Erythromicin
20.	1246	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

21.	1258	*Enterococcus faecalis*	Daptomycin, Teicoplanin, Benzylpenicillin, Ciprofloxacin, Erythromycin, Levofloxacin Vancomycin, Tetracycline
22.	1266	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
23.	1344	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
24.	1379	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
25.	1458	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
26.	1468	*Staphylococcus aureus*	Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
27.	1536	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Tetracycline, Levofloxacin
28.	2822	*Staphylococcus aureus*	Teicoplanin, Vancomycin, Tetracycline, Trimethoprim, Benzylpenicillin, Oxacillin, Erythromycin
29.	2982	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
30.	2997	*Escherichia coli*	Meropenem, Gentamicin, Ciprofolxacin, Trimethoprim,

			Amoxicillin, Piperacillin, Cefuroxime, Ceftriaxone, Cefoperaxone, Cefepime, Ertapenem, Imipenem, Amikacin
31.	3001	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
32.	3054	*Pseudomonas aeruginosa*	Gentamicin, Cyprofloxacin, Levofloxacin, Colistin, Cefeazidime, Aztreonam, Imipenem, Meropenem, Amikacin
33.	3069	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,

Primary antimicrobial screening of endophytic actinobacteria

Actinobacteria isolates were subjected primary antimicrobial screening 33 clinical pathogens, as detailed in Table 1. The screening was done using Agar well diffusion method given by Saadoun and Muhana in 2008. The plates were incubated at a temperature of 37°C for 24 hours without inverting the plates, creating optimal conditions for the growth of both the test organisms and any potential inhibition zones that would indicate antimicrobial activity.

Extract preparation

The selected actinobacteria, following an initial antimicrobial screening, were carefully inoculated into 700 mL of ISP1 medium, which was adjusted to a pH of 7.2 to promote optimal growth. This process was performed in a 500 mL conical flask, ensuring all procedures were carried out under sterile conditions to avoid contamination. The flasks were placed on a shaker incubator with a speed of 120 rpm, allowing for efficient aeration and mixing, and were kept at room temperature for a

duration of one week. To extract the bioactive secondary metabolites, the resulting broth was combined with an equal volume of solvent, utilizing a 1:1 ratio (v/v) of methanol, ethyl acetate, and dichloromethane. This mixture was allowed to sit for 24 hours, facilitating the extraction process. Following this period, the solvents were carefully filtered out using Whatman filter paper. The excess solvent from the filtrate was then evaporated under reduced pressure using rotary evaporator.

Secondary antimicrobial screening of endophytic actinobacteria

The selected actinobacterial extracts were subjected to secondary antimicrobial screening against 15 strains of clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Table 2) pathogens using agar well diffusion method. A concentration of 20 mg/mL of the crude extract was used for this screening and 4% methanol solution serving as a negative control. (Boyanova, 2005). The plates were incubated at 37°C for 24 hours, without inverting them. The diameters of the inhibition zones were subsequently measured in millimeters.

Table 2: Test organism for secondary antimicrobial screening

S.no	Pathogens	Organisms	Resistance
1.	038	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
2.	161	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
3.	365	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin
4.	452	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin
5.	622	*Staphylococcus aureus*	Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Levofloxacin

			Rifampicin, Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
6.	645	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
7.	790	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
8.	809	*Staphylococcus aureus*	Levofloxacin
			Rifampicin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
9.	924	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
10.	1098	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
11.	1246	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
12.	1266	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
13.	1344	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
14.	1379	*Staphylococcus aureus*	Levofloxacin
			Vancomycin, Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin,
15.	1458	*Staphylococcus aureus*	Levofloxacin

Characterization of potent strains using 16S rRNA gene sequencing

Genomic DNA was isolated and purified using a DNA extraction kit (Invitrogen) according to the manufacturer's protocol. Ribosomal RNA (16S rRNA) genes were amplified using universal bacterial primers 16SP1 and 16SP2 as forward and reverse primer (forward 16s rRNA primer 5'- GTGCCAGCAGCCGCGG- 3' and reverse 16s rRNA primer 5'-TACGGYTACCTTGTTACDACTT- 3'). The reactions and conditions of the PCR were performed in a Veriti thermal cycler (Applied Biosystem, Singapore) in a total volume 25µl and the PCR conditions are as follows: initial denaturation step at 94◦C for 5 min, followed by 35 cycles of denaturation at 94◦C for 1 min, annealing at 62◦C for 1 min and extension at 72◦C for 1.2 min with a final extension step at 72◦C for 5 min and sequencing was done commercially at Eurofins Pvt. Ltd. Bangalore, India. Sequences were compared with the reference strains of actinobacteria from the NCBI genomic database using a BLASTn search tool to determine similarity percentages. The strains with the highest similarity percentages were obtained from the NCBI database.

Morphological identification using gram staining and Scanning Electron Microscope

Gram staining was done according to the manufacture's protocol (Kumar et al., 2010). The spore chain morphologies of the isolates were examined using a scanning electron microscope (SEM). The mycelial structures were observed with a phase contrast microscope (Olympus). The organisms were identified according to the 9th edition of Bergey's Manual of Determinative Bacteriology

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay

The DPPH assay was conducted following the methodology described by Villano et al. (2007). An extract without DPPH served as the blank, while ascorbic acid was used as the positive control and methanol as the negative control. The IC_{50}, which denotes the concentration of antioxidant required to achieve a 50% reduction in DPPH, was calculated using a calibration curve based on linear regression. Results were expressed as the percentage reduction of DPPH absorption compared to the control. The percentage of DPPH reduction was calculated using the following equation:

% Scavenging = (Abs of control-Abs of sample)/ Abs of sample *100

Where Abs of sample is the absorbance of the test sample and Abs of control is the absorbance of the control.

ABTS radical scavenging assay

The ABTS+ radical scavenging capacity of the extract was measured using the 96-well microtiter plate method (Re et al., 1999). The absorbance was measured at 734 nm. An extract without ABTS served as the blank, ascorbic acid was used as the positive control, and methanol served as the negative control.

The percentage of and ABTS+ radical scavenging was calculated by using the equation:

% Scavenging= ($A_{control}$-A_{sample})/ A_{sample} X100

Where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

Determination of Total Phenolic Content

Total phenolic content was determined by using the Folin Ciocalteu assay (Singleton 1965). Absorbance was then taken at 725 and 760nm. TPC was calculated from the following equation:

TPC=C X V/M

Where,

TPC= Total phenolic content mg/g of extracts as GAE

C= concentration of gallic acid established from the calibration curve in mg/mL

V= volume of the extracts solution in mL

M= weight of the extracts in gram

Determination of Total Flavonoid Content

The aluminium chloride colorimetric assay method was used for quantification of the total flavonoid content of the methanolic extracts (Zhishen et al., 1999). TFC was calculated from the following equation:

TFC=C X V/M

Where,

TFC= Total flavonoid content mg/g of extracts as QE

C= concentration of quercetin established from the calibration curve in mg/mL

V= volume of the extracts solution in mL

M= weight of the extracts in gram

MIC determination using plate-based method

The determination of the minimum inhibitory concentration (MIC) using the plate-based method employed the agar well diffusion technique. In this process, concentrations of 1, 2, 4, 6, 8, 10, 12, 14, and 16 mg/mL were prepared and the plates were incubated for 24 hours at a consistent temperature of 28 °C and the zone of inhibition was measured after the incubation

MIC determination using broth microdilution method

The determination of the Minimum Inhibitory Concentration (MIC) was conducted using the broth microdilution method, in accordance with the protocol established by Eloff in 1998. Methanolic extracts of the actinobacterial strains were prepared and diluted to various concentrations (1, 2, 4, 6, 8, 10, 12, 14, and 16 mg/mL) to evaluate their antimicrobial activity against bacterial cultures in microtiter plates. Control plates containing no treatment were included for comparison. The plates were incubated at 28 °C for 24 hours, after which absorbance was measured at 700 nm using a UV-Vis spectrophotometer (MultiscanTM GO, Thermo Scientific, MA, USA). The effective concentration for 50% inhibition (EC50) was calculated using GraphPad Prism.

Compound separation using Thin Layer Chromatography (TLC)

Fraction was done using TLC based on polarity (Shoun *et al*., 1998). In which four different types of solvent system were used namely:

solvent system 1: (Butanol-Acetic acid-Water, 30:20:70 v/v)

solvent system 2 (Chloroform-Methanol, 24:1 v/v)

solvent system 3 (Ethyl acetate-Methanol, 6:4 v/v)

solvent system 4 (Hexane-Ethyl acetate, 1:9 v/v)

TLC was carried out on commercially available aluminum TLC sheets (20 × 20 cm with 0.2 mm thickness, silica gel GF254, Merck, Darmstadt, Germany). The solvent fronts were marked and Rf values were measured using the formula,

Retention factor (Rf)= distance travelled by the solute/ distance travelled by the solvent.

Antimicrobial validation of active compounds against MDR isolates

The selected potent compounds underwent testing against multidrug-resistant clinical isolates utilizing the agar well diffusion assay, as described by Saadoun and Muhana in 2008. In this process, pathogenic microbes were carefully inoculated onto a nutrient agar plate. A thin-layer chromatography (TLC) plate, which contained the isolated compounds, was then gently positioned on top of the inoculated media. The assembled plates were incubated at a stable temperature of 28 ± 2°C for a duration of 24 hours, and the zone of inhibition was observed after 24 hours. To ensure reliable results, all experiments were done in triplicates.

Gas Chromatography Mass Spectroscopy analysis

For the gas chromatography-mass spectrometry (GC–MS) analysis, a Clarus 690 Perkin Elmer gas chromatograph was coupled with a Turbomass Gold 5.1 mass spectrometer. An Elite 1 capillary column, made of 100% dimethyl polysiloxane and measuring 123.5 m x 0.678 mm, was used to identify the volatile organic compounds (VOCs) in the methanolic extracts of *Streptomyces* sp. The instrument was set to 40ºC, then raised to 150ºC at 10ºC per minute for 5 minutes. It was subsequently increased to 250ºC at 40ºC per minute and held for 8 minutes. The injection port temperature was set at 250ºC, and the helium flow rate was maintained at 1.5 mL/min. An ionization voltage of 70 eV was used with a 10:1 split mode for sample injection. The mass spectral scanning range was set from m/z 500 to 800. The ion source and interface temperatures were maintained at 230ºC and 240ºC, respectively. The mass spectrometry (MS) data collection started at 3 minutes and ended at 31 minutes, with a solvent cut time of 3 minutes. The spectra of the volatile compounds detected through GC-MS were compared and matched with the NIST17 online library, version 2.339.

High Performance Thin Layer Finger print profile

The extract underwent further analysis utilizing the CAMAG HPTLC instrument, which was paired with the winCAT software for data management. To prepare for the analysis, a stock solution was created by dissolving 50 mg of the sample in distilled water to achieve a concentration of 50 mg/mL. For the chromatography process, TLC plates measuring 2 x 10 cm and coated with 0.2 mm silica gel from Merck, Germany, were selected for the experiment. The sample was carefully spotted onto the plates using the Linomat 5 automated sample spotter from CAMAG, which employed a 100 µl Hamilton syringe for precise application. Three solvent systems were utilized as mobile phase designed specifically for profiling phenolics, glycosides, and anthracenes, ensuring that the separation was effective for each compound type. To facilitate the drying of the bands, nitrogen gas was applied to the plates during the process. The plates, once loaded with the extracts, were placed in a TLC twin trough developing chamber filled with the chosen mobile phase. After the development was complete, the plates were thoroughly dried and subsequently scanned using a TLC scanner with the winCAT software for analysis. Following the drying process, the plates were directly visualized under UV light at wavelengths of 254 nm and 366 nm, allowing for the detailed examination and documentation of the unique fingerprint profiles of the extracts. Key data points, including peak displays, peak densitograms, and peak tables, were meticulously recorded to ensure comprehensive analysis and interpretation of the results.

RESULTS and DISCUSSION

Selection and collection of medicinal plants

A comprehensive literature review led to the selection of five traditional medicinal plants native to the state of Mizoram i.e. *Tithonia diversifolia*, *Centella asiatica* (L.) Urb., *Mikania micrantha*, *Mirabilis jalapa* L. and *Clerodendrum colebrookianum* Walp. Samples of the plants were collected from the surrounding arears of Mizoram University Campus, Aizawl, Mizoram.

Isolation of Endophytic Actinobacteria:

Five nutritional media were used i.e. starch casein agar (SCA), actinomycetes isolation agar (AIA), tyrosine agar medium (ISP7), glycerol asparagine agar base (ISP5), and King's medium. From this, a total of 54 distinct actinobacterial isolates were successfully obtained from five traditionally recognized medicinal plants found in the region of Mizoram. 15 isolates were isolated from *Centella asiatica*. 11 isolates from *Mikania micrantha*, while *Tithonia diversifolia* contributed 6, and 4 isolates from *Clerodendrum colebrookianum* Walp. Among the various media tested, the highest levels of actinobacterial growth were observed in ISP7, followed by SCA, AIA, ISP5, and King's medium. Each individual isolate was arranged using a systematic numbering system, labeled as MMSK1, MMSK2, MMSK3, and so forth, all the way up to MMSK54. The relative abundance of actinobacteria found in *Mirabilis jalapa*, *Centella asiatica*, and *Mikania micrantha* may be attributed to their growing patterns, particularly their proximity to the soil. This closeness seems to foster a favourable environment for the development of endophytic communities, playing a crucial role in the overall health and resilience of these medicinal plants

Primary antimicrobial screening of endophytic actinobacteria

A total of 54 endophytic actinobacteria isolates were subjected to primary antimicrobial screening against 33 clinical multidrug-resistant (MDR) pathogens. The primary screening results revealed that 32 of the 54 isolates exhibited significant inhibitory effects against at least three or more of the tested pathogens. 15 isolates with the most promising inhibition for further investigation.

Extract preparation

15 isolates with the most effective antimicrobial properties from the primary antimicrobial screening were selected for the preparation of extracts. The extracts were prepared using methanol as the solvent, resulting in an approximate yield of 1 to 2 grams of extract from each of the chosen isolates.

Secondary antimicrobial screening of endophytic actinobacteria

20mg/mL of the crude extract against 15 MRSA (Methicillin resistant *Staphylococcus aureus*) clinical pathogens. All of the isolates have antimicrobial properties against atleast to 6 of the tested pathogens. Among these isolates, MMSK12 and MMSK47 emerged as the most promising candidates, exhibiting the ability to inhibit 11 of the tested pathogens. The zone of inhibition for these isolates varied significantly, ranging from 4mm to 16mm in diameter. MMSK12 and MMSK47 were selected for further analysis. to support our research, Bhakyashree and Kannabiran (2020) have also successfully isolated the strain *Streptomyces* sp. VITBKA3, which demonstrates notable antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). This finding highlights the potential of *Streptomyces* sp. is a valuable candidate for developing new antimicrobial agents in the fight against resistant bacterial infections.

Morphological identification using gram staining and Scanning Electron Microscope

Both MMSK12 and MMSK47 were identified as gram-positive bacteria, the intricate details of their spore surfaces, which appeared notably smooth and were adorned with branched, filamentous structures when viewed under Scanning Electron Microscope.

Characterization of potent strains using 16S rRNA gene sequencing

MMSK12 and MMSK47 were subjected to molecular identification using 16S rRNA gene sequencing, MMSK47 was identified as *Streptomyces* sp. with 99.3% genetic similarity to *Streptomyces* sp. NSC30, (MG833381). Similarly, isolate MMSK12 was also classified within the Streptomyces genus, demonstrating the same high percentage of 99.3% similarity with *Streptomyces* sp. QJSt7 (KX950906). The sequences obtained from these molecular identifications have been deposited in the NCBI GenBank database, with accession numbers assigned as OR896092 for MMSK47 and OR896079 for MMSK12. The identification of MMSK12 and MMSK47 as belonging to the genus Streptomyces may provide insight into their notable antimicrobial properties. This is significant because Streptomyces is renowned for being one of the largest and most prolific producers of antibiotics among microbial species.

ABTS radical scavenging assay

In this assay the antioxidant activity of MMSK12 and MMSK47 were measured through the calculation of the IC50 value, which represents the concentration of the extract needed to neutralize or eliminate 50% of the free radicals present in the ABTS solution. The IC_{50} value for the MMSK12 extract was determined to be 82.78 µg/mL, while the MMSK47 extract exhibited an IC_{50} value of 94.14 µg/mL.

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay

The antioxidant potential of MMSK12 and MMSK47 was evaluated by examining their ability to scavenge DPPH free radicals, the results indicated that the DPPH scavenging activity of both MMSK12 and MMSK47 was not as distinct when compared to the values obtained from the ABTS assay. MMSK12 (*Streptomyces* sp.) and MMSK47 (*Streptomyces* sp.), exhibited a low activity against the DPPH radical, yet demonstrated high effectiveness against the ABTS radical. It is essential to highlight that both ABTS and DPPH radicals are structurally different, this disparity leads to varying reactivities. For instance, certain organic compounds, particularly dihydrochalcones and flavanones, do not engage with DPPH radicals; however, they readily interact with ABTS radicals (Platzer et al., 2021).

Determination of Total Phenolic Content

The phenolic content of the MMSK12 and MMSK47 extracts was quantified using Gallic acid as the reference standard. To ensure accuracy, carefully prepared samples were subjected to analysis in relation to a pre-established standard curve utilizing UV-Vis Spectroscopy. The results of this analysis indicated that the MMSK12 extract contains a notable concentration of 68.6±0.51 mg of Gallic Acid Equivalent (GAE) per gram, while the MMSK47 extract has a slightly lower but still significant level of 60.4±0.77 mg of GAE per gram.

Determination of Total Flavonoid Content

A standard curve, previously prepared and securely stored for future applications in UV-Vis spectroscopy, was utilized to evaluate the flavonoid content present in the

methanol extracts of the MMSK12 and MMSK47 samples. This analytical procedure yielded precise measurements of flavonoid concentrations, recorded at 10.92±0.64 mg of Quercetin (QE) per gram of the extract for MMSK12 and 12.89±0.73 mg of Quercetin (QE) per gram for MMSK47.

MIC determination using plate-based method

The minimum inhibitory concentration (MIC) of the methanolic crude extract from MMSK12 and MMSK47 was evaluated against five clinical multidrug-resistant *Staphylococcus aureus* (MRSA) pathogens (365, 790, 924, 1098, and 1246), with the MIC determination concentrations ranging from 1 mg/mL to 18 mg/mL. The concentration of 20 mg/mL was omitted as it was utilized for secondary antimicrobial testing. The MIC zone of inhibition for MMSK12 was recorded at 2 mg/mL against two pathogens (924 and 1246) while it was observed at 4 mg/mL and 12 mg/mL for pathogen 1098 and 790 respectively. In contrast, the MIC zone of inhibition for MMSK47 was found at 1 mg/mL for pathogens 365, 924, 1098, and 1246, and for pathogen 790, it was noted at 2 mg/mL.

MIC determination using broth microdilution method

In the broth microdilution method employed for this study, different concentrations ranging from 1 mg/mL to 18 mg/mL was used. For the isolate MMSK12, the most impressive antimicrobial activity was observed against pathogens 924, 1098, and 1246 with EC_{50} value of 2 mg/mL. EC_{50} of 5 mg/mL and 6 mg/mL was observed in pathogen 365 and 790 respectively. On the other hand, the isolate MMSK47 demonstrated even greater potency, particularly evident in its remarkable activity against pathogens 365, 924, 1098, and 1246, all of which had an EC_{50} value of just 1 mg/mL and EC_{50} value of 2mg/mL was observed for pathogen 790. Our findings indicate that the broth dilution method consistently yields superior results, with notably lower MIC values. This suggests that the broth dilution technique is more efficient in providing precise measurements, as it produces results in decimal form, a capability that plate-based methods lack, where values are only reported as whole numbers.

Fractionation of compounds using Thin Layer Chromatography (TLC)

Among the four solvent systems tested, one particular system was selected to achieve separation. The determined R_f value for MMSK12 was 0.54, indicating its migration distance in the chromatographic process, while MMSK47 exhibited an R_f value of 0.59.

Antimicrobial validation of active compounds against MDR isolates

The antimicrobial validation of active compounds against MDRSA pathogens was done using a plate-based method. The results observed after 24 hours have shown a clear zone on the TLC plates where the separated compounds are present. This validates the antimicrobial activity of the different separated compounds.

Gas Chromatography Mass Spectroscopy analysis

The methanol extract of *Streptomyces* sp. MMSK12 revealed a diverse number of 20 volatile organic compounds, each displaying a wide range of area percentages that varies from a mere 0.324% to 25.57%. Among these compounds, 1-Nenylcycloheptane emerged as the most abundant, dominating the composition of the extract with its significant presence. Following closely in prevalence was decanoic acid, which also contributed substantially to the overall profile of the extract. The methanolic crude extract of *Streptomyces* sp. MMSK41 was analyzed and revealed a total of 19 volatile organic compounds (VOCs). These compounds were detected within a retention time range of 15 to 29 minutes, indicating a diverse metabolic profile. The detailed area percentages of these VOCs demonstrate varying levels of abundance. Notably, 1H-indole-3-acetic acid, specifically identified as 5-chloro-2-methyl-1-(trimethylsilyl) silane, exhibited the highest area percentage at 10.111%. In contrast, Perylene was found to be the least abundant VOC, with an area percentage of only 0.835%. This variation in VOC composition highlights the potential biochemical diversity of *Streptomyces* sp. MMSK47 and its possible applications in various fields, including biochemistry and pharmaceuticals. Key compounds found in MMSK12 and MMSK47 such as N-Decanoic acid (Yang et al., 2023; Ravichandiran et al., 2019), 1,4-Naphthoquinone(Wu et al., 2020; Huang et al.,2011), 2-hexanoic acid (Aranega-Bou et al., 2014; González et al., 2021), Anthracene (Kim et al., 2009; Popiołek 2021), Acetohydrazine (Boulebd et al., 2022; Küçük et al.,2011), 1,3-

Dioxolane (Raskildina et al., 2024; Wood and Szewczak, 2007), and 3-Octanal (Matos et al., 2019; Desai et al., 2013) were found to exhibit remarkable antimicrobial and antioxidant capabilities

High Performance Thin Layer Finger print profile

Three optimized solvent system for mobile phase namely ethyl acetate: methanol: water (20:3:2), cyclohexane: ethyl acetate: formic acid (4:6:1) and toluene: ethyl acetate: methanol: acetic acid (3:5:1:0.5) to detect glycosides (SS1), phenols (SS2) and anthracene (SS3) respectively at a volume of 8 µl of methanolic extract performed at UV 254 nm and 366 nm in visualizer and visible light and single brown spots were detected. The images were captured and scanned in CAMAG TLC Scanner using VisionCATS 3.2 SP2 software. The peak display, peak densitogram, and peak table were recorded and analysed and R_f values were measured then the presence of positive result was recorded on 254 nm and 366 nm. For MMSK47, 5 peaks were recorded in SS1 and SS3 whereas, 7 peaks were recorded in SS2 at 254nm. The peaks with Rf value and area (%) for MMSK47. For MMSK12, 8 peaks were recorded in SS1 and SS2 and 5 peaks were recorded for SS3.

CONCLUSION

The urgent need for discovering and developing new medications has become more critical due to the issues related to treating and managing diseases caused by multiple drug-resistant (MDR) strains of MRSA. The unique and diverse endophytic actinobacteria found in unexplored environments present exceptional opportunities for discovering drugs and therapeutic agents. In addition to their medical applications, the bioactive compounds produced by endophytic actinobacteria are beneficial for agricultural purposes, as they can enhance plant growth, protect against phytopathogens, insects, and pests, improve nutrient absorption, and help sustain crop productivity during extreme abiotic stress conditions such as salinity, drought, and waterlogging.

The current research has revealed that two strains of Streptomyces, designated as MMSK12 and MMSK47, have been successfully isolated from the traditional

medicinal plants *Centella asiatica* and *Mirabilis jalapa*. These plants are indigenous to Mizoram, a region recognized as part of the Indo-Burma biodiversity hotspot, characterized by its rich variety of flora and fauna. Both MMSK12 and MMSK47 have demonstrated significant antimicrobial activity against clinical multidrug-resistant Staphylococcus aureus (MRSA), which poses a critical challenge in treating bacterial infections due to its resistance to many conventional antibiotics. In addition to their antimicrobial properties, MMSK12 and MMSK47 have also exhibited substantial antioxidant activities, suggesting their potential utility in combating oxidative stress-related disorders. Preliminary analysis using gas chromatography-mass spectrometry (GC-MS) have identified several bioactive compounds present in the methanolic crude extract of these strains. However, further investigations are needed to isolate, identify, and quantify the specific bioactive compounds that contribute to the observed antimicrobial and antioxidant effects.

The promising findings associated with *Streptomyces* sp. MMSK12 and MMSK47 imply that these strains could be invaluable sources for the extraction of bioactive compounds. Such compounds have significant implications for the pharmaceutical industry, particularly in the development of novel treatments for infections and diseases linked to oxidative stress. With targeted research and development, there is great potential for these strains to contribute to new therapeutic solutions.