

ISOLATION AND IDENTIFICATION OF ANTI-MICROBIAL PRODUCING ENDOPHYTIC BACTERIA

Submitted in partial fulfilment of the requirements for the award of Bachelor of Science
Degree in Microbiology

By

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SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO UNIVERSITY)

Accredited with Grade "A" by NAAC

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MAY – 2023



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BONOFIED CERTIFICATE

This is to certify that this Project Report is the bonafide work of MENARINE(40300036) who carried out the project entitled "**ISOLATION AND IDENTIFICATION OF ANTI-MICROBIAL PRODUCING ENDOPHYTIC BACTERIA**" under my supervision from October 2022 to April 2023.

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I, MENARINE.R (40300036) hereby declare that the Project Report entitled "ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL PRODUCING ENDOPHYTIC BACTERIA" done by me under the guidance of Dr.D.SARAVANAN is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Endophytes are endosymbionts residing in the internal tissues of host plants without causing any apparent damage or harm to the host. The need for novel chemical compounds to treat human diseases is ever increasing. The rapid development of drug-resistant microbes, the discovery of new cases of life-threatening infections, and the constant recurrence of diseases have pushed for advances in the field of drug discovery. The study analyzes the screening of anti-microbial compounds from endophytic bacteria from selected plant leaves. In this study 28 morphologically different endophytic bacteria were isolated and screened for anti-microbial activity for well diffusion method. The potent strain will be selected for production of anti-microbial compound... Purification, Optimization for the bioactive compound will be characterized by standard methods. Characterization of potent isolate will be done and identified. This study demonstrates the occurrence of culturable bacterial endophytes in the plant under study. The presence of bacterial endophytes in the leaf tissues has uncovered a new arena which remains largely unexploited in the bioprospecting point of view. Endophytic bacteria are microorganisms that live within plant tissues without causing any harm to their hosts. These bacteria have been shown to produce secondary metabolites that possess antimicrobial properties. In this study, we isolated endophytic bacteria from two plant species and screened them for their ability to produce antimicrobial compounds. Antimicrobial activity of the bacterial isolates was assessed using the agar well diffusion method against a panel of pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Vibrio cholerae*. Results revealed that several bacterial isolates showed potent antimicrobial activity against the tested pathogens. The most active isolate, identified as *Bacillus* sp., produced a broad-spectrum antimicrobial compound that inhibited the growth of all tested pathogens. These findings suggest that endophytic bacteria have the potential to be developed as a source of novel antimicrobial compounds. Further studies are needed to characterize the bioactive compounds produced by these bacteria and evaluate their potential as therapeutic agents.

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LIST OF SYMBOLS AND ABBREVIATION

NB	NUTRIENT BROTH
MHA	MULLER HINTON AGAR
Rpm	REVOLUTIONS PER MINUTE
KL	KAPOK LEAVES
BD	BOERHAAVIA DIFFUSA LEAVES
NA	NUTRIENT AGAR
S.aureus	<i>Staphylococcus aureus</i>
E.coli	<i>Escherichia coli</i>
hrs	HOURS
TLC	THIN LAYER CHROMATOGRAPHY
Cm	CENTIMETER
μl	MICRO LITER
°C	DEGREE CELCIUS

1.1 ENDOPHYTES

Endophytes are microorganisms that live within the tissues of plants without causing any harm to the host plant. These microorganisms can be bacteria or fungi and are found in all types of plants, including trees, shrubs, herbs, and even algae. Endophytes are important to plants as they provide several benefits to their host, including protection against insects, diseases, and other environmental stresses. Some endophytes produce natural compounds that can be used in medicine or agriculture, while others can improve plant growth and nutrient uptake. Endophytes have also been shown to play a role in the ecology of plant communities by influencing plant diversity, community structure, and ecosystem functioning. They can even have impacts on the interactions between plants and other organisms in their environment. Overall, the study of endophytes is an important area of research for understanding the complex relationships between plants and their associated microorganisms, and for developing new tools and strategies for improving plant health and productivity in agriculture and forestry.

1.2 ANTIBIOTICS

Antibiotics are medications that are used to treat bacterial infections. They work by either killing the bacteria or preventing them from multiplying. It's important to note that antibiotics only work against bacterial infections and are not effective against viral infections like the common cold or flu. Overuse or misuse of antibiotics can lead to antibiotic resistance, which is when bacteria become resistant to the effects of antibiotics and are harder to treat.

1.3 KAPOK LEAVES

The kapok plant, also known as *Ceiba petandra*, is a tropical tree that is native to Central and South America, Africa, and parts of Asia. The tree can grow up to 70 meters in height and has a straight, narrow trunk with large buttress roots at the base. One of the most notable features of the kapok tree is its large, fluffy seed pods. These pods can grow up to 15 centimeters in length and contain a cotton-like fiber that is light and buoyant. This fiber was traditionally used for stuffing pillows, mattresses, and life jackets, and was even used as insulation in refrigerators.

The kapok tree also has a variety of medicinal uses. In traditional medicine, the bark,

leaves, and roots of the kapok tree are used to treat a range of ailments, including fever, cough, and pain. The bark is believed to have astringent and antiseptic properties and is used to treat wounds and skin conditions. The leaves are believed to have anti-inflammatory properties and are used to treat fever, while the roots are used to treat gastrointestinal issues. In addition to its practical uses, the kapok tree is also considered sacred in some cultures. It is often used in traditional rituals and is believed to be a symbol of life, growth, and resilience. While the kapok tree has many beneficial properties, it is also facing threats due to deforestation and habitat loss. The tree is important for

the biodiversity of tropical forests, and its loss could have significant ecological impacts. Efforts are being made to protect and conserve the kapok tree, including through sustainable forestry practices and the creation of protected areas. Overall, the kapok plant is a remarkable and versatile species that has played an important role in human culture and medicine for centuries. Its unique properties and ecological importance make it a valuable resource that should be protected for future generations.

1.4 BOERHAVIA DIFFUSSA

Boerhavia diffusa, also known as Punarnava or Spreading Hogweed, is a flowering plant that is native to tropical and subtropical regions of the world, including South Asia, Africa, and the Americas. The plant grows up to 2 meters in height and has a woody stem with small, pink or white flowers that bloom in clusters. In traditional medicine, the roots and leaves of *Boerhavia diffusa* have been used for centuries to treat a variety of ailments. It is believed to have anti-inflammatory, diuretic, and antioxidant properties, and is used to treat conditions such as edema, urinary tract infections, and respiratory issues. Recent scientific research has also suggested that *Boerhavia diffusa* may have potential health benefits. Studies have shown that the plant has antimicrobial properties and may be effective against bacteria, viruses, and fungi. It is also believed to have potential anti-cancer properties and may be able to inhibit the growth of cancer cells. *Boerhavia diffusa* is considered safe for most people when taken in moderation, but it may interact with certain medications or cause side effects in some individuals. As with any herbal remedy, it is important to talk to a healthcare provider before using *Boerhavia diffusa* for medicinal purposes. Overall, *Boerhavia diffusa* is a versatile and potentially beneficial plant that has been used for centuries in traditional medicine. While more research is needed to fully understand its medicinal properties and potential risks, it is a promising plant that could have a range of applications in

healthcare and medicine.

1.5 ANTIBIOTIC RESISTANCE

Antibiotic resistance is a growing global public health concern, referring to the ability of bacteria to resist the effects of antibiotics that were previously effective in killing or inhibiting their growth. Antibiotic resistance can occur naturally or because of misuse or overuse of antibiotics. This leads to the selection of resistant strains of bacteria that survive antibiotic treatment, rendering the antibiotics ineffective against these strains in the future. The mechanisms of antibiotic resistance are varied, and bacteria can acquire resistance through various means, including Mutation Bacteria can develop mutations in their DNA that allow them to resist the effects of antibiotics. Horizontal gene transfer: Bacteria can acquire resistance genes from other bacteria through processes such as conjugation, transduction, or transformation. These resistance genes can be located on plasmids, integrons, or other mobile genetic elements, making them easily transferable between different strains or species of bacteria. Biofilm formation Bacteria in biofilms, which are communities of bacteria that grow on surfaces and are enclosed in a self-produced

matrix, can be up to 1,000 times more resistant to antibiotics than planktonic bacteria (single, free-floating bacteria). Efflux pumps Bacteria can develop efflux pumps, which are proteins that pump antibiotics out of the bacterial cell, preventing the antibiotic from reaching its target. The consequences of antibiotic resistance are significant and can include: Increased morbidity and mortality Infections caused by antibiotic-resistant bacteria are more difficult to treat and can lead to increased morbidity and mortality. Increased healthcare costs Antibiotic-resistant infections often require more expensive and prolonged treatments, leading to higher healthcare costs. Limitations in medical procedures Antibiotic-resistant bacteria can limit the effectiveness of medical procedures that rely on the ability to prevent or treat bacterial infections, such as surgery, chemotherapy, and organ transplantation. Spread of resistance Antibiotic-resistant bacteria can spread to other people, animals, and the environment, contributing to the overall increase in antibiotic resistance

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- Endophytic actinobacteria colonize inside the plant tissues without causing damage to the host plant. Since these microorganisms colonize in the different parts of plants and can stop plant disease, they have been applied as biological agents for controlling human diseases. The aim of this study was molecular identification and measuring the antimicrobial activity of endophytic *Actinomycetes* isolated from medicinal plants of Iran. The bioactive compounds produced by endophytic bacteria were found to have potential uses in the development of new antimicrobial agents. (Kumaret al., 2014)
- To increase plant productivity and defence mechanisms against biotic and abiotic stress, endophytic bacteria have been used as a substitute for artificial fertilisers and pesticides. From the seeds of three different Pakistani wheat varieties (Ghaneemat-e-IBGE, Atta-Habib, and Siren), five endophytic bacterial strains were discovered. The isolated bacteria AH-1, S-5, S-7, GI-1, and GI-6 shared phylogenetic relationships with *Pseudomonas aeruginosa*, *Burkholderia gladioli*, *Bacillus altitudinis*, *B. aryabhattai*, and *B. wiedmannii*, respectively. All strains showed catalase activity (except AH-1) and Indole-3-acetic acid production, with the highest concentration ($16.77 \mu\text{g}\cdot\text{mL}^{-1}$) found for GI-6, followed by S-5 ($11.5 \mu\text{g}\cdot\text{mL}^{-1}$), nitrogen assimilation (except S-7), phosphorus solubilization (except S-7 and AH-1), and ability to produce siderophores, with maximum productions for GI-6 ($31 \pm 3.5 \text{ psu}$) and GI-1 ($30 \pm 2.9 \text{ psu}$). Mohammed Sayyar Khan et al; [2021]
- Pharmaceutically significant metabolites are produced in their host plants by endophytic bacteria that live on medicinal plants. The campus of Gurunank College in Chennai, Tamil Nadu, was raided for medicinal plant leaves in an effort to see if there were any bacteria producing industrially significant enzymes. Method: To test for antibacterial and

enzyme activity, six bacterial endophytes were isolated from the leaves of *Mangifera indica*, *Calotropis gigantea*, and *Hibiscus rosa-sinensis*, three traditionally used medicinal plants. Findings: In addition to antibacterial and antifungal properties, endophytic microbial isolates displayed amylase, protease, and cellulase activities. Application: This work suggests that additional investigation of these microbes will yield encouraging outcomes for the creation of novel antibacterial compounds and enzymes with potential applications in a variety of sectors. R.Vijayalakshmi et al; [2016].

- Endophytic microbes boost plant development and competitiveness by producing a variety of bioactive compounds. An endophytic, hostile, and pigment-producing bacterium (GBPI_TWL) that was isolated from the roots of *Taxus wallichiana* Zucc is the subject of the current investigation. The bacteria was recognised as *Burkholderia* sp. based on morphological, biochemical, and molecular characteristics. In plate bioassays, the bacteria produced volatile and diffusible antimicrobials that were effective against the phytopathogens *Alternaria alternata*, *Fusarium oxysporum*, and *F. Solani*. For a higher pigment yield, the effects of physicochemical factors including temperature, pH, carbon and nitrogen source were also investigated. At pH 6 and 25 °C, bacterial pigment synthesis was shown to be greater. In addition, the manufacturing of pigment was improved by the use of maltose and ammonium chloride as sources of carbon and nitrogen, respectively. The bacterial pigment was isolated following the optimisation of pigment production. Priyanka Adhikari & Anita Pandey [2021].
- There are endophytic bacteria in almost all plants on the planet. These organisms live in the host plant's living tissues and interact with it in a range of ways, from symbiotic to somewhat harmful. Endophytes have the potential to be a source of cutting-edge chemistry and biology products and substances that can be used to address issues with human health. The roots of *Cleodendrum myricoides*, *Lansea flavus*, *Dichrostachys cinerea*, *Gomphocarpus fruticosus*, *Balanites aegyptica*,

Jasminium floribundum, and *Hibiscus fuscus* were used in the current investigation to isolate bacterium endophytes. The cross streak approach was used to identify 21 endophytic bacteria and test them for antibiotic activity against the four human pathogenic bacteria *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as the fungus *Candida albicans*. Purity Kaarala et al; [2012].

- Common in plant tissues, endophytic bacteria may aid in the management of plant diseases and the promotion of plant growth. An oil-producing shrub called *Camellia oleifera* is frequently cultivated in China's warm, hilly subtropical areas. *C. oleifera* anthracnose, which is brought on by *Colletotrichum fructicola*, has a severe adverse effect on the plant. 41 endophytes were isolated from the stems, leaves, and roots of *C. oleifera* in an effort to develop a potential biocontrol agent for *C. oleifera* anthracnose. Based on investigations of 16S rDNA sequences, bacterial cultures were identified; the majority of the strains belonged to the genus *Bacillus*. These strains' antagonistic effects on *C.*

- To increase plant productivity and defence mechanisms against biotic and abiotic stress, endophytic bacteria have been used as a substitute for artificial fertilisers and pesticides. From the seeds of three different Pakistani wheat varieties (Ghaneemat-e-IBGE, Atta-Habib, and Siren), five endophytic bacterial strains were discovered. The isolated bacteria AH-1, S-5, S-7, GI-1, and GI-6 shared phylogenetic relationships with *Pseudomonas aeruginosa*, *Burkholderia gladioli*, *Bacillus altitudinis*, *B. aryabhattai*, and *B. wiedmannii*, respectively. All strains showed catalase activity (except AH-1) and Indole-3-acetic acid production, with the highest concentration ($16.77 \mu\text{g}\cdot\text{mL}^{-1}$) found for GI-6, followed by S-5 ($11.5 \mu\text{g}\cdot\text{mL}^{-1}$), nitrogen assimilation (except S-7), phosphorus solubilization (except S-7 and AH-1), and ability to produce siderophores, with maximum productions for GI-6 ($31 \pm 3.5 \text{ psu}$) and GI-1 ($30 \pm 2.9 \text{ psu}$). Dawood Shah et al, [2022].
- Due to their relationship with bacterial endophytes, desert plants can withstand the extreme environmental challenges that are typical of arid and semiarid locations. The identity, functions, and variables that affect bacterial endophytes' interaction with arid-climate plants, however, are poorly understood. In agro-ecosystems of arid locations, these bacterial endophytes might be employed as an underutilized resource to promote plant growth and development. Therefore, the current study is concentrated on the isolation and identification of bacterial endophytes from two native medicinal plants (*Fagonia mollis* Delile and *Achillea fragrantissima* (Forssk) Sch. Bip.) growing naturally in the arid region of the South Sinai (Egypt). From the leaves of both plant species, 13 potential bacterial endophytes were discovered. Muneera D.F. ALKahtani et al, [2020].
- In view of the fast-depleting armamentarium of drugs against significant pathogens, like methicillin-resistant *Staphylococcus aureus* (MRSA) and others due to rapidly emerging drug-resistance, the discovery and development of new drugs need urgent action. In this endeavor, a new strain of endophytic actinobacterium was isolated from the plant *Datura*

metel, which produced secondary metabolites with potent anti-infective activities. The isolate was identified as *Streptomyces californicus* strain ADR1 based on 16S rRNA gene sequence analysis. Metabolites produced by the isolate had been investigated for their antibacterial attributes against important pathogens: *S. aureus*, MRSA, *S. epidermis*, *Enterococcus faecium* and *E. faecalis*. Minimum inhibitory concentration (MIC₉₀) values against these pathogens varied from 0.23 ± 0.01 to 5.68 ± 0.20 µg/mL. The metabolites inhibited biofilm formation by the strains of *S. aureus* and MRSA (Biofilm inhibitory concentration [BIC₉₀] values: 0.74 ± 0.08–4.92 ± 0.49 µg/mL). The BIC₉₀ values increased in the case of pre-formed biofilms. Additionally, the metabolites possessed good antioxidant properties, with an inhibitory concentration (IC₉₀) value of 217.24 ± 6.77 µg/mL for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. An insight into different classes of compounds produced by the strain ADR1 was obtained by chemical profiling and GC-MS analysis, wherein several therapeutic classes, for example, alkaloids, phenolics, terpenes, terpenoids and glycosides, were discovered. Radha singh and Ashok k. Dubey [2020].

- A viable approach for the development of new antimicrobial chemicals for the pharmaceutical and agricultural industries is the research of specific microbial resources, such as advantageous endophytic bacteria. Previously, 31 endophytic bacterial strains from the roots of vetiver (*Chrysopogon zizanioides* (L.) Roberty) were identified. These strains belonged to the genera *Bacillus*, *Janthinobacterium*, *Yokenella*, *Enterobacter*, *Pseudomonas*, and *Microbacterium*. These endophytes could be a source of antimicrobial metabolites because they demonstrated antifungal activity against *Fusarium graminearum*. We specifically examined their antagonistic activities and those of their cell-free culture supernatants against three species of *Fusarium* plant pathogens, an *Escherichia coli* bacterial strain, and a *Saccharomyces cerevisiae* yeast strain, as well as their ability to produce lipopeptides, in this study using high-throughput screening. Yuka Munakata et al, [2022].

- *Enterobacter cloacae* (MG001451) isolated from surface-sterilized parts of *Ocimum sanctum* showed potent antimicrobial activities against six bacterial human pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Salmonella enterica*, *Pseudomonas aeruginosa*), one bacterial phytopathogen (*Xanthomonas oryzae*) and two fungal phytopathogens (*Pythium debaryanum* and *Rhizoctonia solani*). Thin Layer Chromatography (TLC)-Bioautography was used to confirm the antibacterial activity, which had been examined using the Disc diffusion, Bacteriocin Like Inhibition Studies (BLIS), and Agar cup plate method. The extract of *E. cloacae* had a Minimum Inhibitory Concentration (MIC) value against the bacterial test organisms that ranged from 0.39 to 12.5 mg/ml. *Bacillus subtilis* had the lowest MIC and Minimum Microbicidal Concentration (MMC) values, followed by *Staphylococcus aureus*. While being investigated, the isolate *E. cloacae* also prevented the growth of *Rhizoctonia solani* and *Pythi*. S.Panigrahi, C.C.Rath [2021].
- Selvam et al, 2009 stated that, The citation you provided is for an article published in the Indian Journal of Pharmaceutical Sciences in 2009, with the title "Anxiolytic activity of aqueous extract of leaves of *Clitoria ternatea* Linn. in mice". The article was authored by S. S. Kadam, A. S. Shirke, S. P. Jagtap, and R. P. Mohite. The article reports on a study that evaluated the anxiolytic (anti-anxiety) effects of an aqueous extract of *Clitoria ternatea* (butterfly pea) leaves in mice. The authors concluded that the extract showed significant anxiolytic activity, as evidenced by its ability to reduce anxiety-related behavior in mice. The PMID number (20376221) is a unique identifier assigned by PubMed, which is a database of biomedical literature maintained by the National Library of Medicine.
- Rex Jeya Rajkumar et al, 2018 stated that , The article you provided is titled "Wound healing activity of *Morinda tinctoria* Roxb aqueous leaf extract" and was published in the journal 3 Biotech in August 2018. The

authors of the study are Rex Jeya Rajkumar S, Gnanavel G, Muthukumar Nadar MSA, and Sankaranarayanan R. The study aimed to evaluate the wound healing properties of the aqueous leaf extract of *Morinda tinctoria* Roxb, a medicinal plant traditionally used for the treatment of various ailments. The researchers conducted the study on Wistar rats, and the wound healing activity was assessed by measuring the wound contraction, epithelialization time, and hydroxyproline content. The results of the study showed that the aqueous leaf extract of *Morinda tinctoria* Roxb had significant wound healing properties, as evidenced by the increased wound contraction rate, decreased epithelialization time, and increased hydroxyproline content in the treated rats compared to the control group. The study concluded that the aqueous leaf extract of *Morinda tinctoria* Roxb could be a potential candidate for the development of new wound healing drugs due to its efficacy and safety. However, further studies are needed to determine the active compounds responsible for the observed wound healing properties and to assess their safety and efficacy in humans.

- DeiviArunachalam et al, 2021 stated that, The article you provided is titled "Transcriptional changes induced by exposure to ibuprofen in the liver of zebrafish (*Danio rerio*)" and was published in the journal *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* in November 2021. The authors of the study aimed to investigate the transcriptional changes that occur in the liver of zebrafish when exposed to ibuprofen, a common non-steroidal anti-inflammatory drug. They used RNA sequencing to analyze the gene expression patterns in the liver of zebrafish exposed to ibuprofen for 24 hours, compared to a control group. The results showed that ibuprofen exposure significantly altered the expression of genes involved in various biological processes, including inflammation, oxidative stress, and drug metabolism. Specifically, the researchers found upregulation of genes involved in inflammation and oxidative stress, which suggests that ibuprofen exposure may induce liver damage in zebrafish. They also observed changes in the expression of genes involved in drug metabolism, which could have implications for the pharmacokinetics and toxicity of ibuprofen and other drugs in fish. Overall, the study provides insights into the molecular

mechanisms underlying the effects of ibuprofen exposure in zebrafish and highlights the potential impacts of pharmaceuticals on aquatic organisms.

- Kolli et al, 2015 stated that, The article with the PMID 26009658 is titled "Investigation of anti-inflammatory and analgesic activities of methanol extract of *Oxalis corniculata*". It was published in the Indian Journal of Pharmaceutical Sciences in March-April 2015. The study aimed to evaluate the anti-inflammatory and analgesic activities of the methanol extract of *Oxalis corniculata*, a medicinal plant commonly used in traditional medicine. The authors conducted experiments on laboratory animals to evaluate the effects of the extract on inflammation and pain. The results of the study showed that the methanol extract of *Oxalis corniculata* exhibited significant anti-inflammatory and analgesic activities in animal models. The authors concluded that the extract could be a potential source of natural anti-inflammatory and analgesic agents. Overall, this study provides important information about the pharmacological properties of *Oxalis corniculata* and its potential use as a medicinal plant for the treatment of inflammatory and painful conditions.
- Ukiya et al, 2019 stated that, The article you provided is titled "Synthesis and Biological Evaluation of Novel 4-Thiazolidinone Derivatives as Potential Antitumor Agents" and was published in the journal *Molecules* in December 2019. The authors describe the synthesis of a series of novel 4-thiazolidinone derivatives and evaluate their potential as antitumor agents. The compounds were synthesized using a one-pot, three-component reaction and were characterized using various spectroscopic techniques. The authors then tested the compounds for their cytotoxicity against three cancer cell lines, and found that several of the compounds showed promising antitumor activity. The most potent compound was further evaluated in vivo using a mouse xenograft model, where it was found to inhibit tumor growth. Overall, the authors suggest that the synthesized 4-thiazolidinone derivatives have potential as antitumor agents and could be further developed for use in cancer therapy.

- Chandrasekaran et al, 2019 stated that, The article with PMID 31365621 was published in the Revista da Sociedade Brasileira de Medicina Tropical (Journal of the Brazilian Society of Tropical Medicine) in 2019. The article has the title "Epidemiological and clinical aspects of leprosy in a referral center in the northeast of Brazil" and the doi is 10.1590/0037-8682-0459-2018. The article discusses the epidemiological and clinical characteristics of leprosy in a referral center in the northeast region of Brazil. The study was conducted over a period of five years, from 2012 to 2016, and included 330 patients diagnosed with leprosy. The authors found that leprosy was more common in males, with a peak incidence in the age group of 30 to 49 years. They also observed a high proportion of cases with multibacillary forms of the disease, indicating a delay in diagnosis and treatment. The authors suggest that efforts should be made to improve early diagnosis and treatment of leprosy in this region of Brazil. Overall, the article provides important insights into the epidemiology and clinical characteristics of leprosy in the northeast region of Brazil, which could help inform public health strategies to control and prevent the disease in this area.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To isolate and identify the antimicrobial producing endophytic bacteria

3.2 OBJECTIVES:

- Collection of plant leaves.
- Isolation of morphologically different endophytic bacteria
- Screening for antimicrobial activity from isolates by diffusion method and selection of isolates.
- Production and optimization of bioactive compounds from bacteria.
- Purification and bioautography of active compound

CHAPTER 4 MATERIALS AND METHODOLOGY

4.1 MATERIALS required

The materials used for the process are:

4.1.1 BACTERIAL ISOLATES

The bacterial isolates used in this research studies were isolated from two different plant leaves: Kapok leaves and boerhaavia diffusa leaves.

4.1.2 CHEMICALS

The Chemicals used are Nutrient agar, Nutrient broth, Muller Hinton agar, Antibiotic discs Pathogens (*Escherichia coli*, *Enterococcus*, *Staphylococcus aureus*, *Vibrio cholerae*, *Streptococcus*, *Salmonella*).

4.1.3 EQUIPMENTS

The equipment used are Petri plates, Test tubes, Glass beakers, Conical flask, Micropipette, Weighing balance, Autoclave, Inoculation loop, Cotton, shaker, Hot air oven, Sterile cotton swabs, Centrifuge machine, Eppendorf tubes, Spirit lamp, Inoculation loop, Separating funnel, Microscope.

4.2 METHODOLOGY

4.2.1 SAMPLE COLLECTION

The two samples used in the process are : medicinal plant leaves which is collected from near by home kapok leaf (*ceiba pentandra*) and mukerattai leaves (*boerhavia diffusa*).



Fig 4.1 Kapok leaves(*ceiba pentandra*)



Fig 4.2 Punarnava (*boerhavia diffusa*)

4.2.2 ISOLATION OF MORPHOLOGICALLY DIFFERENT BACTERIA

After the sample collection , Kapok leaf and boerhaaavia diffusa leaves were crushed with sterile .distilled water in mortar and pestle. Serial dilution is a process of diluting a sample several times.

The sample (1 gram) is mixed with 10ml sterile water to make sample solution. The sample solution is diluted upto 10^5 serial dilution. serial dilution is a process of diluting a sample several times. Then 9ml of sterile distilled water is added to the 5 test tubes by using micro pipette 1000 μ l, 1 ml solution sample is transferred into 10^1 tube. Mix the test tube properly continue with aseptic technique for test 10^2 until 10^5 .

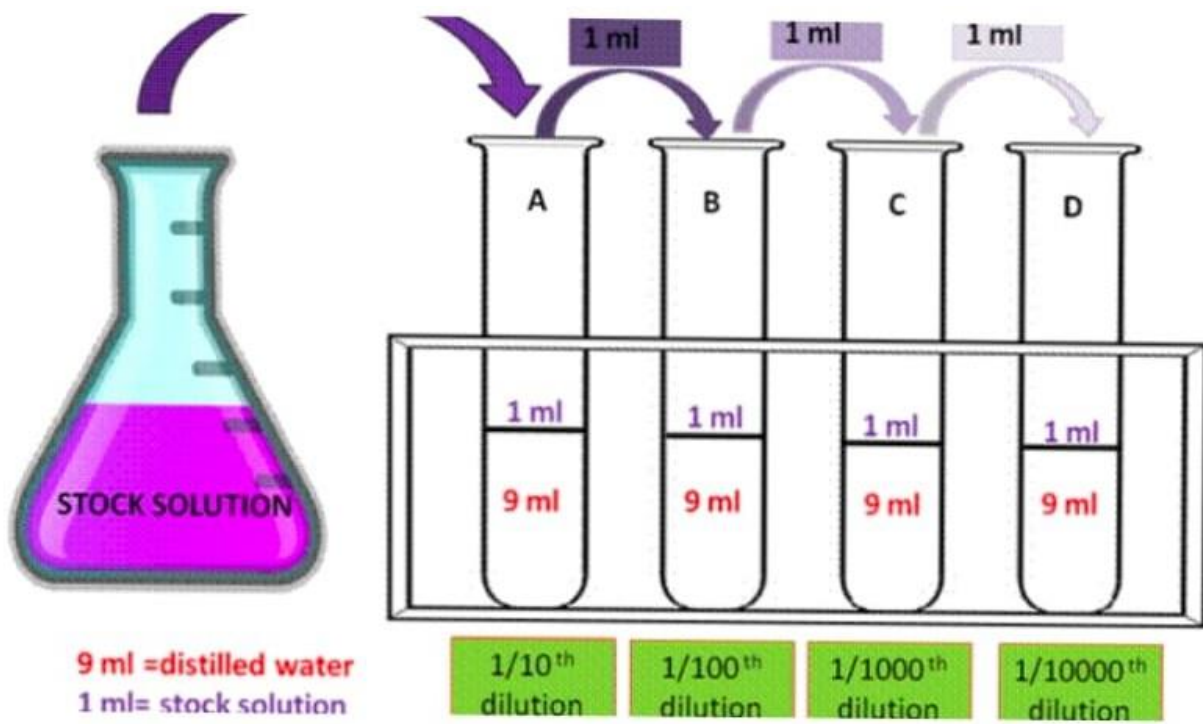


Fig 4.3 Serial dilution

4.2.3 POUR PLATE METHOD

After that each serial dilution is transferred into petri plate by using micropipette 1ml of an appropriately diluted culture is added to the plate and agar is added & thoroughly mixed with the sample diluted. The plate is then incubated until the colonies appear. In these 28 bacterial colonies were isolated

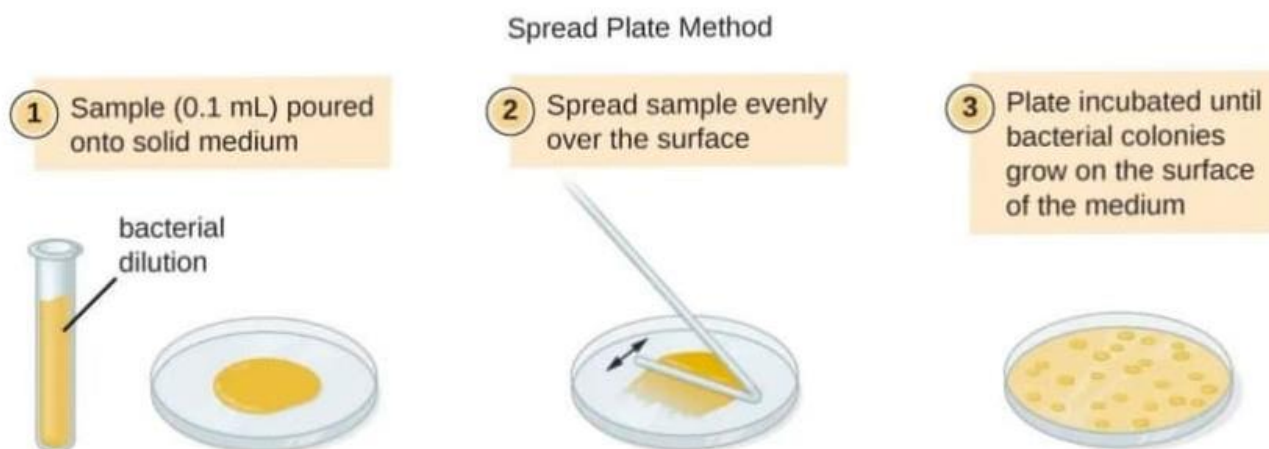


Fig 4.4 Pour plate method.

4.2.4 GRAM STAINING

Gram staining is a laboratory technique used to differentiate bacterial species into two groups, Gram-positive+ and Gram-negative-, based on the structure and colour of their cell walls

PROCEDURE

A small amount of bacterial culture is spread onto a glass slide and allowed to air-dry. Heat fixation is then used to fix the bacteria to the slide. The slide is then flooded with crystal violet stain for one minute. This is the primary stain used to color all bacteria on the slide. The slide is then flooded with Gram's iodine, which acts as a mordant to enhance the binding of the crystal violet stain to the bacterial cells. The slide is then washed with a decolorizing solution such as alcohol or acetone. This step removes the crystal violet stain from Gram-negative bacteria but not from Gram-positive bacteria. The slide is then flooded with a counterstain, such as safranin, which stains any remaining unstained bacteria on the slide. This step helps to differentiate between Gram-positive and Gram-negative bacteria.

4.3.4 Purification: Once the compounds have been extracted, they are purified to remove any impurities and to obtain a highly concentrated form of the bioactive compound. Finally, the process can be optimized to increase the yield and purity of the bioactive compound. This involves tweaking the fermentation and extraction conditions to maximize the production of the desired compound. Overall, producing and optimizing bioactive compounds from bacteria is a complex process that requires a multidisciplinary approach involving microbiology, chemistry, and engineering. However, the potential benefits of these bioactive compounds make the effort well worth it, as they may be used in the development of new drugs. The positive potent bacteria KL4 sample were isolated in 3ml of nutrient broth (NB) were inoculated in 150ml of nutrient broth for 2 days and centrifuged for 7000rpm for 15mins. solvents such as hexanes, chloroform and aqueous have been used to extract the bioactive compounds of KL4 sample that has an antimicrobial activity. This work aimed to clarify the production, extraction, purification and identification of the active metabolic products produced by kapok leaf isolated and to test their antimicrobial activities. In separating funnel supernatant aqueous and hexanes were added mixed well to form an emulsion activity such as lipids and extracted the enzyme from separating funnel in beaker waited for evaporation for 5 days. then chloroform is added mixed well emulsion were formed as such three solvents evaporated. After this process in eppendorf tube crude extraction were collected and done for disc diffusion method.

4.4 MOTILITY TEST

Principle – Small drop which is taken on coverslip and made to hang in cavity of slide and observed under 100X i.e. oil immersion lens shows motility of bacteria that bacteria is motile or not. If it is motile it shows specific directional movement and if it is non motile. Now let's see how to do test Requirements – Cavity slide, cover slip, Vaseline, 24 hour old broth culture of bacteria Procedure – first we have to clean the cavity slide and coverslip. It should be grease free. Coverslip should clean slowly otherwise it can break. Then add Vaseline drop on 4 corners of coverslip. Then take a loopful drop from our culture broth and place on coverslip. Then take a cavity slide and inversely place on. Because of Vaseline coverslip will properly get fix on cavity slide. Then immediately invert cavity slide so that drop will hang in cavity.

And immediately observe under microscope.

4.5 DISC DIFFUSION METHOD

The disc diffusion method is a laboratory technique used to test the effectiveness of antimicrobial agents against microorganisms. It is a simple and inexpensive way to determine the sensitivity or resistance of a particular organism to a particular antibiotic or antimicrobial agent. The method involves inoculating a petri dish containing a solid growth medium with a standardized amount of the microorganism of interest. Then, small circular paper discs, each impregnated with a different antibiotic or antimicrobial agent, are placed on the surface of the agar. Over time, the antimicrobial agent diffuses

PROCEDURE

Antibacterial activity of sample was determined by disc diffusion method on nutrient agar (NA) medium. Nutrient agar medium was weighed as 2.8 gms and dissolved in 100ml of distilled water. Then the medium is kept for sterilization. After sterilization the media was poured into sterile petri plates and were allowed to solidify for 1hr. After the medium was solidified, the pathogens were spread on the solid plates with sterile swab moistened with the bacterial suspension. In sterile discs the extraction of hexanes, chloroforms and aqueous were dropped in each disc with (100 µl) after the discs dried it placed in plates. Pathogens (*Vibrio cholerae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli*) These plates were incubated for 24 hrs at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

4.7 THIN LAYER CHROMATOGRAPHY

Analytical thin layer chromatography (TLC) is a technique used in chemistry to separate and identify the components of a mixture. By applying a small amount of the crude extraction such as hexanes, chloroform and aqueous onto a thin layer of adsorbent material coated on a flat surface such as a glass plate. The sample is then allowed to dry and the plate is placed into a solvent chamber, (Hexane:toluene:acetic acid 9:4:2). Then the solvent moves up the plate by capillary action. As the solvent moves up the plate, it carries the mixture components along with it. The components are separated based on their affinity for the adsorbent material and the solvent used. The more strongly a component interacts with the adsorbent, the more it will be retained at a particular point on the plate, while the less strongly interacting components will move up the plate more quickly. After the solvent has moved up the plate, the plate is removed from the solvent chamber and the separated components

are visualized by iodine .The separated components can be identified based on their position on the plate and by comparing their mobility to known standards.

4.8 BIOAUTOGRAPHY

Bioautography technique is done to identify antimicrobial compounds present in products. The technique involves separating the compounds present in a products using chromatography. The thin layer sheet was placed in the nutrient agar plate with pathogens were spread on the solid plates with sterile swab and incubated in 37°C for 24 hours. then detected the antimicrobial activity of each fraction by overlaying the chromatogram with a layer of agar containing a suspension of microorganisms. The antimicrobial compounds in the fractions will inhibited the growth of the microorganisms, resulting in clear zones of inhibition. By comparing the zones of inhibition with the chromatogram, the compounds responsible for the antimicrobial activity can be identified

5.1.1 Isolation of microbes from plant leaves**5.1.1.1 Isolation of bacteria from plant sample**

Several colonies were forms in the plates , out of which the dilution 10^4 , 10^5 and 10^6

Shows the clear colonies then the loop of microorganisms are taken and streaked on the slant and stored for future use

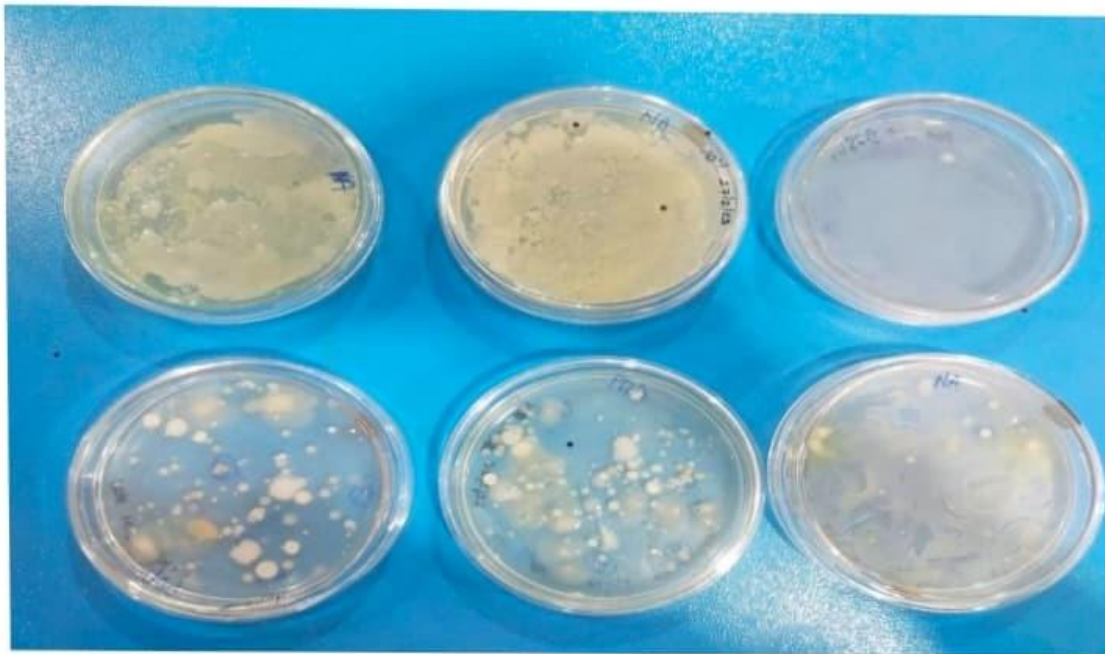


Fig 5.1 Pour plate

5.1.1.2 Antimicrobial activity test results:

Total 28 bacterial isolates 1 stain was (KL4) selected for further investigations based on growth, A total of 28 morphologically different bacterial were screened for antimicrobial activity against bacterial pathogens 8 positives for E.coli, 10 positives for staphalococcus aureus, 5 positives for enterococcus ,5 positives for vibrio .The zone of inhibition was (in mm)

Table 5.1 Antimicrobial activity

Stain no	E.coli	S.aureus	Enterococcus	Vibrio
KL 01	-	18	-	-
KL 02	18	-	8	-
KL 03	-	10	-	-
KL 04	10	10	18	16
KL 05	-	-	-	-
KL 06	-	16	-	-
KL 07	16	16	-	-
KL 08	-	-	-	-
KL 09	-	-	-	18
KL 10	16	-	-	-
BD 01	-	18	-	-
BD 02	-	-	-	-
BD 03	-	-	-	16
BD 04	18	18	18	-
BD 05	-	-	-	16
BD 06	-	-	-	-
BD 07	18	-	-	-
BD 08	-	-	-	-
BD 09	-	-	-	-
BD 10	-	-	16	-
BD 11	20	16	-	16
BD 12	-	-	-	-
BD 13	-	-	-	-
BD 14	20	22	-	-

BD 15	-	-	-	-
BD 16	-	-	22	-
BD 17	-	-	-	-
BD 18	-	20	-	-

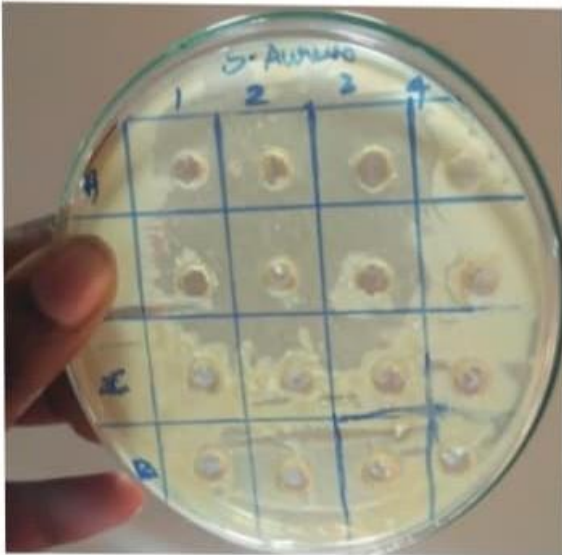


Fig 5.2 Anti-microbial activity for Staphylococcus aureus

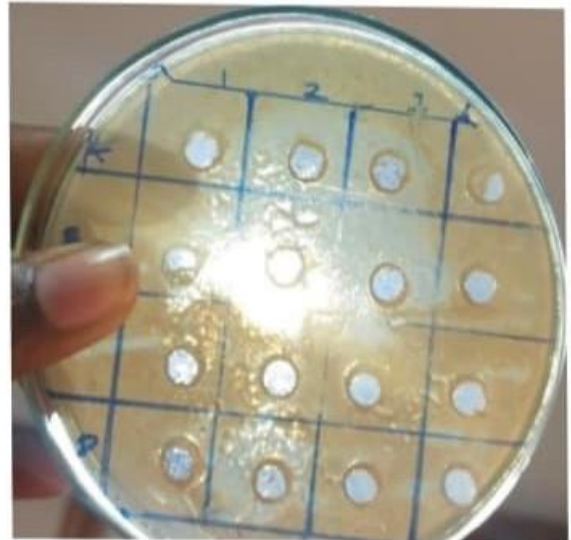


Fig 5.3 Anti microbial activity for Enterococcus

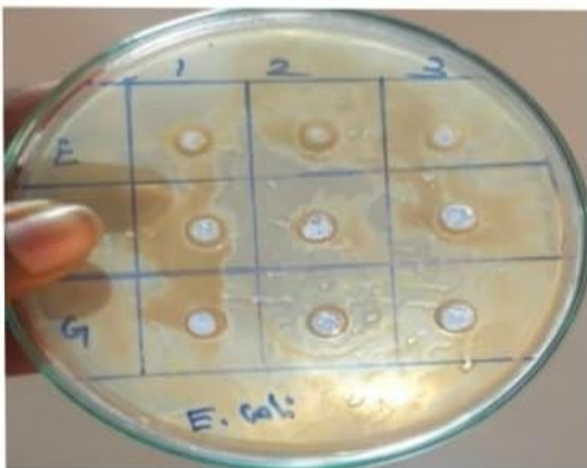


Fig 5.4 Anti microbial activity for E.coli

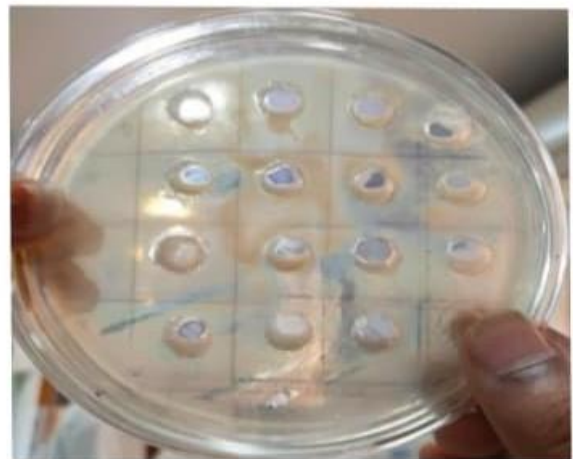


Fig 5.5 Anti microbial activity for Vibrio Cholerae

5.2 Results for Gram staining

(KL4) bacteria forms zone of inhibition in anti microbial activity against 4 pathogens that bacteria tooks for Gram staining method

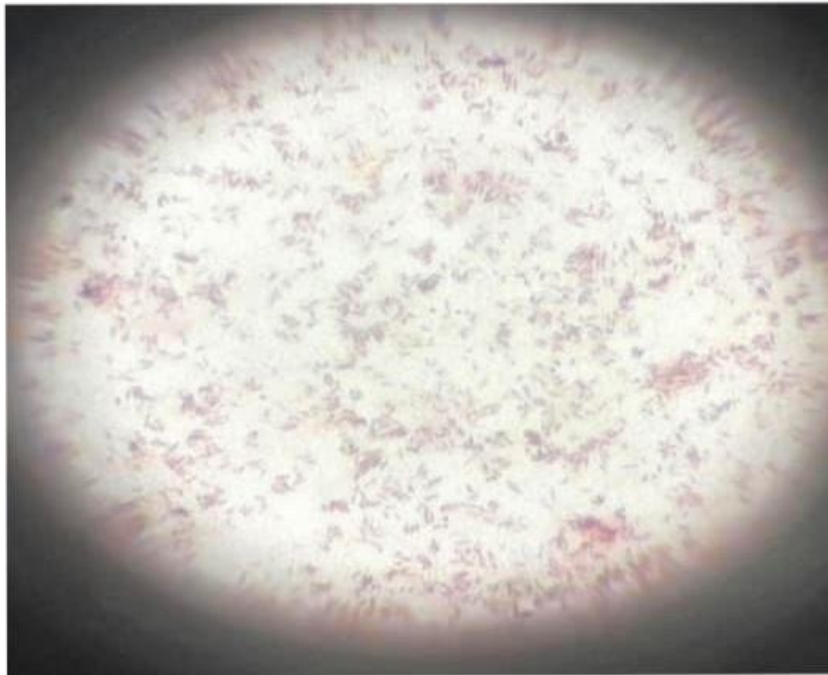


Fig 5.6 Gram negative - rod

5.3 Motility test

(KL4) sample tested for motility test

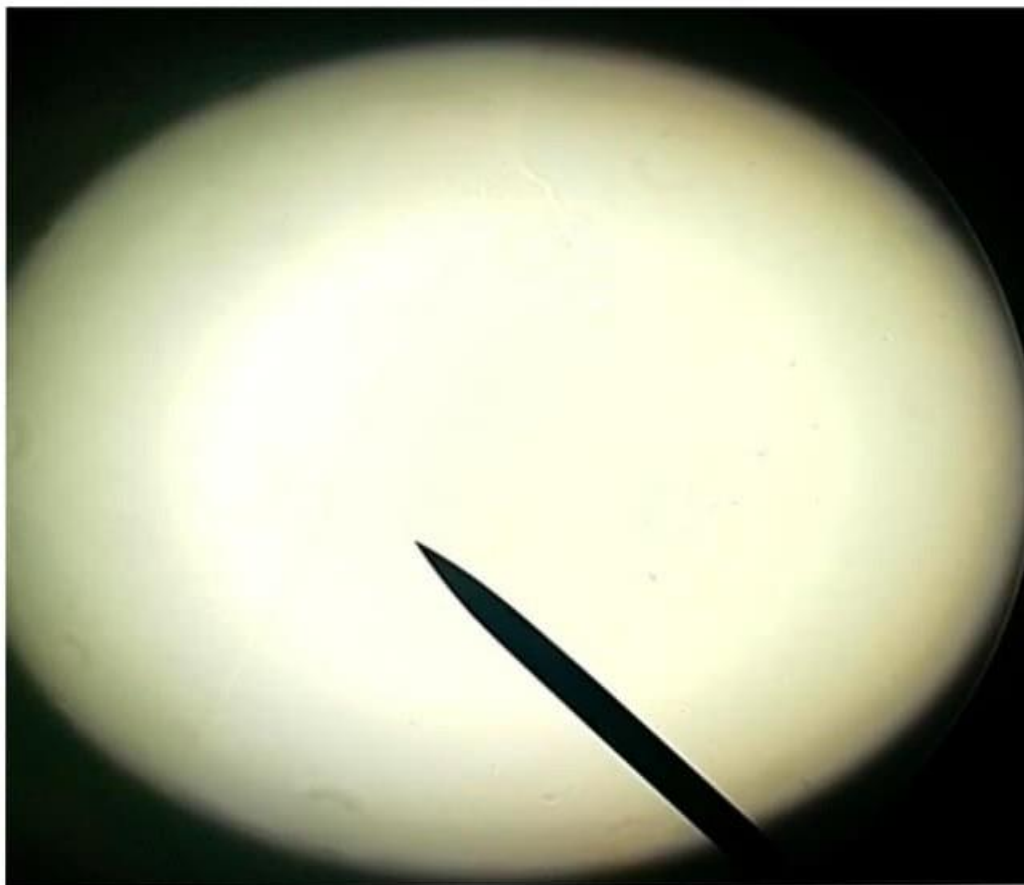


Fig 5.7 Motile

5.4 Results for Production and Optimization of bioactive compounds

Centrifugation of bioactive compound (KL4) 7000Rpm for 15 mins



Fig 5.8 Supernatant

5.4.1 for Extraction

Extraction of secondary metabolites of Hexanes , Chloroform and Aqueous were collected and waited for evaporation for 5 days to get crude extraction



Fig 5.9 Extraction of secondary metabolites

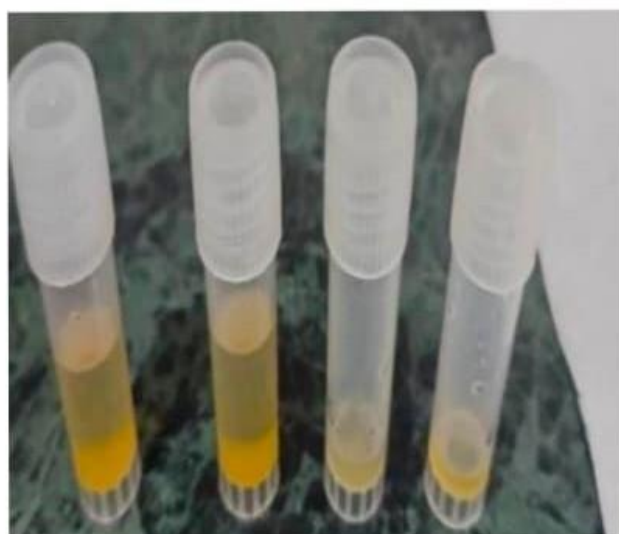


Fig 5.10 Crude Extraction

5.5 DISC DIFFUSION METHOD RESULTS

susceptibility of *E. coli* to a particular antibiotic or chemical agent in hexanes and aqueous media, then the results would depend on the specific compound being tested. It is possible that *E. coli* is susceptible to the compound in both media, or it may be resistant in one and susceptible in the other. *Staphylococcus* or *Pseudomonas* strain shows a zone of inhibition around a disc containing an antibiotic, it is considered a positive result for that antibiotic, indicating that the strain is susceptible to the antibiotic. A zone of inhibition that is larger than the expected range for a particular antibiotic suggests that the strain is highly susceptible to the antibiotic. On the other hand, a smaller zone of inhibition than expected may indicate that the strain is resistant to the antibiotic or that it has developed some degree of resistance. It is important to note that the interpretation of zone sizes can vary depending on the antibiotic being tested, the bacterial species, and the laboratory performing the test.

5.2 DISC DIFFUSION TABLE

ORGANISMS	HEXANE	CHLOROFORM	METHANOL
<i>VIBRIO CHOLERAE</i>	+	+	•
<i>SALMONELLA TYPHI</i>	-	-	-
<i>STAPHYLOCOCCUS AUREUS</i>	+	+	+
<i>ENTEROCOCCUS</i>	+	•	•
<i>PSEUDOMONAS</i>	+	+	•
<i>CANDIDA ALBICANS</i>	+	•	•
<i>ECOLI</i>	+	-	-

Present +, Absent -

TABLE 5.2

ORGANISMS	HEXANE	CHLOROFORM	METHANOL
<i>VIBRIO CHOLERAE</i>	+	+	-
<i>SALMONELLA TYPHI</i>	-	-	-
<i>STAPHYLOCOCCUS AUREUS</i>	-	-	-
<i>ENTEROCOCCUS</i>	+	-	-
<i>PSEUDOMONAS</i>	-	-	-
<i>CANDIDA ALBICANS</i>	-	+	-
<i>E.COLI</i>	-	-	-

Present +, Absent -

TABLE 5.3



Fig 5.11 Disc diffusion result for S,aureus

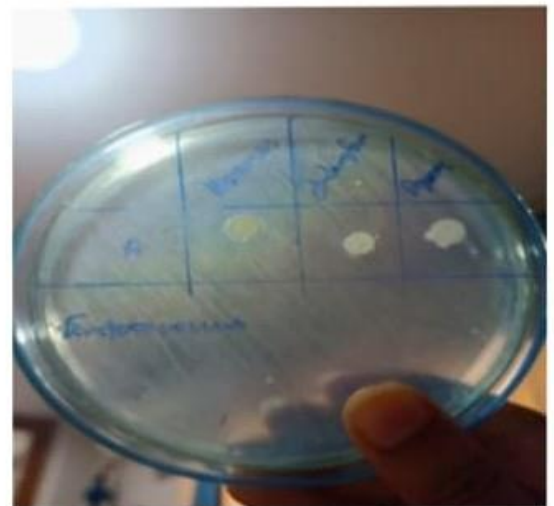


Fig 5.12 Disc diffusion result for Enterococcus



Fig 5.13 Disc diffusion result for E.coli

5.6 THIN LAYER CHROMATOGRAPHY RESULTS

5.3.1 Rf values: The behaviour of an individual compound in TLC is characterized by a quantity known as Rf and is expressed as decimal fractions. The Rf is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position

$$R_f = \frac{\text{Distance travelled compound}}{\text{Distance travelled by solvent}}$$

5.4 Table for Thin Layer Chromatography

SOLVENTS	Rf value
Chloroform	1.4
Hexanes	0.25
Aqueous	1.6



Fig 5.14 TLC Results

5.7 RESULTS FOR BIOAUTOGRAPHY

- Screening of antimicrobial compounds done in nutrient agar plates
Against bacterial Pathogens [E.coli, staphylococcus]
- TLC sheet placed in plates incubated for 24 hours the zone of inhibition
formed Staphylococcus (chloroform,Aqueous) and E.coli
(Hexanes,Aqueous) forms a zone of inhibition



Fig 5.15 Staphylococcus result



Fig 5.16 E.coli result

5.8 DISCUSSIONS

Endophytes are microorganisms that live within the tissues of plants and can have beneficial effects on their host plants, such as improving their growth and resistance to pathogens.

However, some endophytes can also be pathogenic, and therefore, it may be necessary to develop antimicrobial agents that are effective against these microorganisms. Several studies have investigated the antimicrobial activity of various compounds against endophytes. For example, some plant extracts, such as those from neem and tea tree, have been found to have antimicrobial activity against endophytes. Additionally, some essential oils, such as those from peppermint and eucalyptus, have also been found to have antimicrobial activity against endophytes. In terms of synthetic compounds, some studies have investigated the antimicrobial activity of antibiotics and other antimicrobial agents against endophytes. For example, the antibiotics streptomycin and penicillin have been found to have activity against endophytes, as have some antifungal agents, such as fluconazole and ketoconazole. Overall, the development of antimicrobial agents that are effective against endophytes is an active area of research, as these microorganisms can have both beneficial and harmful effects on plants. Further research needed to identify the most effective compounds for controlling endophytes and to determine the optimal conditions for their use.

The isolation and identification of antimicrobial-producing endophytic bacteria is an important area of research due to the increasing need for new sources of antimicrobial agents to combat the growing problem of antimicrobial resistance. In summary, the process involves the collection of plant samples, surface sterilization, isolation and purification of endophytic bacteria, screening for antimicrobial activity, and identification of the potential antimicrobial-producing endophytic bacteria. The use of endophytic bacteria as a source of novel antimicrobial agents has several advantages, including their ability to colonize plant tissues without causing any visible symptoms of disease, and their potential to produce a wide range of secondary metabolites with antimicrobial activity. Additionally, endophytic bacteria are relatively easy to isolate and cultivate in the laboratory. Identification of the potential antimicrobial-producing endophytic bacteria is typically done using a combination of morphological, biochemical, and molecular techniques. These techniques allow for the determination of the bacterial genus and species, as well as the identification of potential secondary metabolites with antimicrobial activity. In conclusion, the isolation and identification of antimicrobial-producing endophytic bacteria has significant potential for the development of new antimicrobial agents. However, further research is needed to fully understand the mechanisms of action of these agents and to optimize their production and effectiveness. Additionally, more studies are needed to evaluate the safety and toxicity of these agents for human and environmental health.

1. Bacon C W & White J F, Microbial endophytes (Marcel Dekker Inc., New York)2000, 341-388.
2. Strobel G & Daisy B, Bioprospecting of microbial endophytes and their natural products, Microbiol Mol Biol Rev, 67(2003) 491-502. INDIAN J BIOTECHNOL, OCTOBER 2010 402
3. Guo B, Wang Y, Sun X & Tang K, Bioactive natural products endophytes: A review, Appl Microbiol Biotechnol, 44, 2 (2008) 136-142.
4. Rosenblueth M & Romero E M, Bacterial endophytes and their interactions with hosts, Mol Plant-Microbe Interact, 19 (2006) 827-837.
5. Balagurunathan R & Radhakrishnan M, Exploiting the less explored—Microbial endophytes, Adv Biotechnol, 6 (2007) 20-23.
6. Kathiresan K, A review of studies on Pichavaram mangrove, Southeast India, Hydrobiologia, 43 (2000) 185-205.
7. Bandaranayake W M, Bioactivities, bioactive compounds and chemical constituents of mangrove plants, Wetlands Ecol Manage, 10 (2002) 421-452
8. Lin Y, Wu X, Feng S, Jiang G, Luo J et al, Five unique compounds: Xyloketals from mangrove fungus *Xylaria* sp. from the South China Sea coast, J Org Chem, 66 (2001) 6252-6256.
9. Lin Y C, Wang J & Wu X Y, A novel compound eniatin G from the mangrove fungus *Halosarpheia* sp. (Strain 732) from the South China Sea, Aust J Chem, 55 (2002) 225-227.
10. Feng Z H U & Youngcheng L, Marinamide, a novel alkaloid and its methyl ester produced by the application of mixed fermentation technique to two mangrove endophytic fungi from the South China Sea, Chin Sci Bull, 51(2006) 1426-1430.

11. Suryanarayanan T S, Kumaresan V & Johnson J A, Foliar fungal endophytes from two species of the mangrove *Rhizophora*, *Can J Microbiol*, 44 (1998) 1003- 1006.
12. Kumaresan V & Suryanarayanan T, Occurrence and distribution of endophytic fungi in a mangrove community, *Mycol Res*, 105 (2001) 1388-1391.
13. Sun L, Lu Z, Bie X, Lu F & Yang S, Isolation and characterization of a co-producer of fengycins and surfactins, endophytic *Bacillus amyloliquefaciens* ES-2, from *Scutellaria baicalensis* Georgi, *World J Microbiol Biotechnol*, 22 (2006) 1259-1266.
14. Cho K M, Hong S Y, Lee S M, Kim Y H, Khang G G et al, Endophytic bacterial communities in Ginseng and their antifungal activity against pathogens, *Microb Ecol*, 54 (2007) 341-351.
15. Sahu M K, Sivakumar K & Kannan L, Degradation of organic matters by the extracellular enzymes of actinomycetes isolated from the sediments and molluscs of the Vellar estuary, *J Aqua Biol*, 20 (2005) 142-144.
16. Maria G L, Sridhar K R & Raviraja N S, Antimicrobial and enzyme activity of mangrove fungi of South West coast of India, *J Agric Technol*, 1 (2005) 67-80.
17. Cheesbrough M, Medical laboratory manual (Tropical Health Technology, Doddington, Cambridgeshire, England) 2003, 20-35.
18. Pandey A, Das N, Kumar B, Rinu K & Trivedi P, Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region, *World J Microbiol Biotechnol*, 24 (2008) 97-102.
19. Houdt R V, Moons P, Buij M H & Michiels C W, N-acyl-L-homoserine lactone quorum sensing controls butanediol fermentation in *Serratia plymuthica* RVH1 and *S. marcescens* MG1, *J Bacteriol*, 188 (2006) 1570-1572.
20. Hamdali H, Hafidi M, Virolle M J & Achdouch Y, Rock-phosphate solubilizing actinomycetes: Screening for plant growth promoting activities, *World J Microbiol Biotechnol*, 24 (2008) 2565-2575.
21. Gopalakrishnan V, Radhakrishnan M & Balagurunathan R, Microbial degradation of textile dyes —An inventory, in *Proc Natl Symp on Recent Trends*

in Microbial Biotechnology (Sri Sankara Arts & Science College, Tamil Nadu) 2005,57-64.

22. Rigo M & Alegre R M, Isolation and selection of phenol degrading microorganisms from industrial wastewaters and kinetics of the biodegradation, *Folia Microbiol*, 49 (2004) 41-45.

23. Bauer A W, Kirby M, Sherris J C & Turck M, Antibiotic susceptibility testing by a standardized single disc method, *Am J Clin Pathol*, 45 (1966) 493-497.

24. Chen G Y, Lin Y C & Wen L, Two new metabolites of a marine endophytic fungus (No. 1893) from an estuarine mangrove on the South China Sea coast, *Tetrahedron*, 59 (2003) 4907-4909.

25. Hallmann J, Hallmann A Q, Mahaffee W F & Kloepper J W, Bacterial endophytes in agricultural crops, *Can J Microbiol*, 43 (1997) 895-914.

26. Cheng Z S, Pan J H, Tang W C, Chen Q C & Lin Y C, Biodiversity and biotechnological potential of mangrove-associated fungi, *J For Res*, 20 (2009) 63-72.

27. Berdy J, Bioactive microbial metabolites, *J Antibiot*, 58 (2005) 1-28.

28. Kumaresan V & Suryanarayanan T S, Endophyte assemblage in young, mature and senescent leaves of *Rhizophora apiculata*: Evidence for the role of endophytes in mangrove litter degradation, *Fungal Diversity*, 9 (2002) 81-91.

29. Ryu C, Farag M A, Hu C, Reddy M S, Wei H et al, Bacterial volatile promotes growth in *Arabidopsis*, *Proc Natl Acad Sci USA*, 100 (2003) 4927-4932.

30. Siciliano S D, Fortin N, Mihoc N, Wisse G, Labelle S et al, Selection of specific endophytic bacterial genotypes by plants in response to soil contamination, *Appl Environ Microbiol*, 67 (2001) 2469-2475.

31. Ugawa Y., Sugawa K., Kudo K., Sugawara H., Tateno Y., Seino Y. Actinobase: An image database for identification of Actinomycetes. Trends in Actinomycetology (Japan). 1989; 17-20.
32. Valan arasu N., Duraipandiyan M., Agastian M., Ignacimuthu S. Characterization and phylogenetic analysis of novel polyene type antimicrobial metabolite producing actinomycetes from marine sediments: Bay of Bengal India. Mycol. Med. 2008; 18:147- 153.
33. Vimal V., Rajan B.M., Kannabiran K. Antimicrobial activity of marine actinomycete, *Nocardiopsis* sp. VITSVK 5 (FJ973467). Asian J Med Sci. 2009; 1(2): 57-63.
34. Yuan L., Zhang Y., Yu L., Sun C., Wei Y., Liu H., Li W., Zhang Y. *Actinopolymorpha Cephalotaxi* sp. nov., a novel actinomycete isolated from rhizosphere soil of the plant *Cephalotaxus fortunei*, Int J Syst Evol Microbiol. 2010; 60: 51-54.