Exploring the Antimicrobial Potential of Mangrove Bacteria Against the Human Pathogen

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COMPLETION CERTIFICATE

This is to certify that the dissertation report " **Exploring the Antimicrobial Potential of Mangrove Bacteria Against the Human Pathogen** " is a bonafide work carried out by **Mr Omkar Ramesh Kamble** under my supervision in partial fulfilment of the requirements for the award of the degree of Master Of Science in the Discipline Marine Microbiology at the School of Earth , Ocean and Atmospheric Sciences , Goa University.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Exploring the Antimicrobial Potential of Mangrove Bacteria Against the Human Pathogen"** is based on the results of investigations carried out by me in Marine Microbiology at School of Earth , Ocean and Atmospheric Sciences, Goa University under the Supervision of Dr. Chanda Berde and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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PREFACE

The research carried out for the dissertation titled "**Exploring the Antimicrobial Potential of Mangrove Bacteria Against the Human Pathogen".** This dissertation delves into the uncharted territory of mangrove bacteria, seeking to unlock their potential in combating human pathogens. Through a series of rigorous experiments and analyses, the antimicrobial capabilities of these bacterial communities are scrutinized offering insights into their role as potential sources of new antibiotics. This study was based on checking the mangrove associated bacteria has the potential to become an antibiotic resistance against the human pathogens. This study was started after sampling sediment and water samples from Ribandar, Goa. Antimicrobial activities have been performed using the mangrove associated bacterial isolates in (Chapter 3 and 4). Isolates having good activity have been tentatively identified as R1, R6, R15, R20 and R32 Mangrove ecosystem harbour diverse bacterial communities, some of which produce compounds with antibiotic properties. These bacteria have adapted to unique environmental conditions, leading to the synthesis of novel bioactive compounds. Researchers are exploring these bacteria foe potential pharmaceutical applications due to their ability to produce antibiotics that could combat drug-resistant pathogens.

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Omkar R. Kamble

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ABBREVIATIONS USED

Entity	Abbreviation
Colony Forming Units Per Millilitres	CFU/mL
Concentrated	Conc.
Degree Centigrade	℃
Deoxyribonucleic acid	DNA
Distilled Water	D.W
Grams	g
Kilometres	km
Litres	L
Microliters	µl
Millilitres	ml
Milligrams per litre	Mg/L
Not Detected	ND
Parts per thousand	% or ppt
Potential of Hydrogen	pH
Room Temperature	RT
Seconds	Sec.
Species	Sp.
Volume	Vol
World Health Organization	WHO
Antimicrobial peptides	APs

Abstract

The antibacterial activity of mangrove bacteria from Chorao island, Goa was investigated against human bacterial pathogens *Staphylococcus aureus, E. coli, Pseudomonas* and *Bacillus*. In this study bacterial identification, antimicrobial assay was carried out using bacterial isolates by using disc method against the human bacterial culture. Antibiotic peptide extraction was also carried out by ammonium sulphate precipitation where precipitate was used against the human pathogens by disc method. The result was shown that in antimicrobial assay among the 27 isolates R1, R6, R15, R20 and R32 were showing largest zone against the human pathogens where isolate R6 shows highest zone of inhibition (more sensitive) against the *Pseudomonas* which it culture whereas isolate R15 shows lowest zone of inhibition (resistance) against *S. aureus*. In the antibiotic peptide extraction isolate R20 shows zone of inhibition against all bacterial cultures which showing potential implications for antibiotic development or clinical use.

CHAPTER 1
INTRODUCTION

The mangrove is a tropical coastal biome situated in the land-to-sea transition zone, dominated by a specific plant species (Zhou et al. 2006). The ecosystem is characterized by periodic tidal flooding, resulting in highly variable environmental factors like salinity and nutrient availability, resulting in unique and specific characteristics (Holguin et al. 2006). The phylogenetic and functional description of microbial diversity in the mangrove ecosystem is not as comprehensive as in other environments (Zhou et al. 2006). Understanding the bacterial diversity and distribution in a mangrove ecosystem can enhance our comprehension of its functionality and microbial interactions (Kathiresan and Sel- vam 2006). The microbiota in mangroves, due to their unique conditions and bacterial adaptation, holds significant potential for biotechnological resource exploitation. The biotechnological potential involves characterizing novel enzymes from previously uncharacterized bacterial species, which could have applications in various aspects of human life, including agriculture and medicine (Lageiro et al. 2007). An estuary is a semi-enclosed coastal body of water that allows free ocean water communication and mixes with freshwater from land (Levinson, 2010). The estuarine system provides a favourable and beneficial habitat for mangrove plant species (Pawar, 2012). The mangrove ecosystem is a highly productive ecosystem that generates commercial forest products, safeguards coastlines, and supports the growth and reproduction of coastal fisheries and aquatic organisms (Kumar and John, 2013). Over 59 mangrove species in India are present, with 19 of these species residing in the estuarine waters of Goa (Mohandas et al 2014).

Mangrove forests are the world's most productive ecosystems, enhancing coastal waters, protecting coastlines, and supporting coastal fisheries. However, they face extreme conditions like fluctuating salinities, tide actions, strong winds, high temperatures, and muddy soils. These plants have highly developed morphological, biological, ecological, and physiological adaptations, making them unique in their ability to adapt to these extreme conditions (Kathiresan et al., 2001). Mangrove environments offer a valuable resource for discovering new microbiota with significant applications in pharmaceutical science (Gayathri et al., 2010). Mangrove, a prevalent species in the Indo-Pacific region, is found in shallow waters along the Indonesian coastal regions (Ariyanto et al., 2018). The Mangrove green belt, while not able to completely prevent an extreme tidal wave, can mitigate its destructive impact (Osti et al., 2009). Mangrove waste, decomposed by bacteria, provides high levels of nutrients in the area. Mangrove extract has shown potential as a multi-drug resistant anti-bacterial, enhancing its health benefits (Pringgenies et al., 2020).

Mangrove forests are crucial coastal resources for socio-economic development, as they provide commercial products, fishery resources, and eco-tourism opportunities. Most coastal

communities rely on these resources for their livelihood, making them vital for the socioeconomic growth of the region (Kathiresan and Bingham, 2001). Mangrove forests are home to over 70 direct human activities, including fuel-wood collection and fisheries (Dixon, 1989). The mangrove ecosystem is a type of wetland ecosystem (Moudingo et al. 2020). Ecotourism is determined by ecological parameters such as mangrove density and mangrove type, which determine the suitability of an area for tourism (Malik et al. 2019). Nugroho et al (2013) stated that mangrove forests play a crucial role in providing nutrients to the organisms that inhabit them. Mangrove species must be capable of adjusting to salinity conditions and drought during periods of receding seawater (Liang et al. 2008). The food chain is crucial in forming ecosystem biomass and explaining the relationships within the ecosystem (Yonvitner et al., 2019). Mangrove forests, despite their potential, are highly susceptible to damage (Takarendehang et al., 2018).Good knowledge and skills can significantly optimize mangrove ecosystems by maintaining and preserving mangrove forests, benefiting the community greatly (Alongi, 2002).The carbon cycle is a crucial part of the biogeochemical cycle (Alongi ,2020).The characteristics of mangrove habitat factors vary across different regions (Poedjirahajoe et al., 2017).The mangrove forest is the most abundant carbon-rich forest with a high organic matter content (Donato et al., 2012). Mangrove ecosystems are the most productive wetlands along tropical and subtropical coastlines, covering approximately 137,760 km2 across 118 countries. They serve as breeding and feeding grounds for various marine and terrestrial species, host a variety of organisms, act as a barrier against tidal currents and natural catastrophic events, and serve as a carbon sink. Mangroves are essential in the global carbon cycle and are crucial in sustaining coastal human populations and their economy.

Mangroves are instrumental in the net export of organic carbon and nutrients to estuarine and coastal waters. The sediments are usually silty and poorly oxygenated, influenced by tidal flushing. This exposes microbes to diverse carbon sources, pH changes, and metal exchange, particularly iron. High nutrient and organic carbon concentrations, high microbial turnover rates, and low porosity prevent gaseous exchanges, resulting in increased biological oxygen demand and reduced bioavailability of iron. Various mechanisms have evolved to overcome this limitation by producing siderophores and siderophore-like organic compounds (Sinha & Bhaskar, 2020). Mangrove ecosystems are crucial for both ecological and economic reasons. They provide food, breeding grounds, and nursery sites for various terrestrial and marine organisms, which are used for human sustainability and livelihoods. They also offer protection against catastrophic events like tsunamis, tropical cyclones, and tidal bores, and can reduce shoreline erosion. Mangrove forests contain a rich biological diversity of plants, animals, and

microorganisms, contributing to the productivity of the ecosystem. Microbes play a critical role in creating and maintaining the biosphere, providing biotechnologically valuable products, recycling nutrients, producing and consuming gases, destroying pollutants, treating anthropogenic wastes, and controlling plant and animal pests. Mangrove microorganisms are a major source of antimicrobial agents and medicinal compounds, including enzymes, antitumor agents, insecticides, vitamins, immunosuppressants, and immune modulators (Somiari et al., 2022). Mangrove forests are coastal environments in tropical and subtropical climates, found in tidal zones where continental freshwater meets oceanic water. These forests are highly productive and play a crucial role in maintaining other coastal environments like coral reefs, despite intense salinity variations throughout the tidal cycle (Taketani et al., 2010). Mangroves are a diverse tropical marine ecosystem that transitions between terrestrial and marine environments, providing ecological functions such as protecting coastlines from erosion, providing habitat for aquatic and terrestrial fauna, and serving as a nursery and spawning ground for commercial fauna like mud crab, fish, and prawn. The ecosystem's moist, muddy soil is rich in organic matter, but it lacks oxygen. The productivity of mangrove vegetation is determined by soil nutrients and the decomposition process carried out by microorganisms like cellulolytic bacteria. Bacteria are directly involved in the nutrient cycle in the soil and play a crucial role in the decomposition of organic matter and mineralization of organic compounds (Dewiyanti et al., 2023). Mangroves are a highly productive marine ecosystem with a complex and variable distribution of microbial activities. Bacteria play a significant role in biomineralization and biotransformation of minerals, degrading leaves and wood provided by mangrove plants. Detritus, rich in enzymes and proteins, forms a large reservoir of carbon and energy for the estuarine food web. Bacteria are major participants in the carbon, sulphur, nitrogen, and phosphorous cycles in mangrove forests. About 30% to 50% of organic matter in mangrove leaves is leachable water-soluble compounds, while the remaining fraction consists of plant structural polymers known as cellulosic substances (Behera et al., 2014).

Mangrove forests are a highly productive ecosystem that enriches coastal waters, yields commercial forest products, protects coastlines, and supports coastal fisheries. They thrive in conditions of high salinity, extreme tides, strong winds, high temperature, and muddy, anaerobic soils. Mangroves, along with their associated microbes, fungi, plants, and animals, form the mangrove forest community or Mangal. They provide nursery habitat for commercial fish, crustaceans, and wildlife species, sustaining local fish and shellfish populations.

Mangrove root systems slow water flow and facilitate sediment deposition, allowing them to adapt to salinity conditions due to their resistance to salt concentration. Many mangroves have aerial stilt roots, which act as anchoring structures to withstand wave action. Some mangroves have inverted wedge-like projections on the ground called pneumatophores, which allow plants to breathe oxygen through the pores during prolonged submergence. Bacterial diversity in mangrove ecosystems is studied worldwide for their unique biochemical processes, including photosynthesis, nitrogen fixation, and methanogenesis. Microorganisms from mangrove ecosystems contain useful enzymes, proteins, antibiotics, and salt-tolerant genes, all of which have biotechnological significance (Tam et al., (2018).

As pathogen load increases, mangrove plants in marine waters may develop anti-bacterial compounds, potentially combating common human pathogens like *S.aureus, E.coli, Klebsiella, Bacillus*, and *Vibrio* sp (Arivuselvan et al 2011).The rise in antibiotic resistance among pathogenic bacteria has led to an increasing therapeutic issue (Sen and Batra, 2012). The World Health Organization (WHO) has deemed antibiotic-resistant bacteria as a significant global health threat. Antimicrobial peptides are small molecules that play a crucial role in the innate immunity of various organisms, including humans, insects, plants, and microorganisms. They are cationic and amphipathic due to their high proportion of arginine, lysine, and hydrophobic residues. The killing mechanism of most antimicrobial peptides is the disruption of membrane organization through depolarization through hydrophobic and electrostatic interaction between the peptides and negatively charged lipids on bacterial cell membrane. Other mechanisms affecting microbial viability include inhibiting DNA, RNA, protein, and cell wall synthesis by targeting essential intracellular factors. Resistance to antimicrobial peptides is unlikely, as microbes need significant changes to their gene sequences, membrane structure, and lipid composition to evade them. As a result, antimicrobial peptides have gained attention for their broad-spectrum activity and low host toxicity, making them a potential therapeutic candidate against drug-resistant bacteria (Ho et al., 2019).

Antimicrobial peptides (APs) are ancient weapons produced by immune responses, involved in the direct destruction of various microorganisms through various mechanisms. They have broad activity spectra against Gram-positive and Gram-negative bacteria, enveloped viruses, fungi, and parasites. As resistance to various antibiotics has developed in various microbes, the potential of APs as novel therapeutic agents is being evaluated. Optimization of APs for therapy should focus on factors like proteolytic degradation and toxicity reduction to mammalian cells.

Strict guidelines should be established to prevent or hinder future bacterial resistance to Aps (Baltzer & Brown, 2011).

Antimicrobial peptides, also known as host defence peptides, are an evolutionarily conserved part of the innate response found in all life classes(Baltzer & Brown, 2011).

Antimicrobial peptides, also known as bacteriocins, are host defence peptides produced by bacteria. They are positive-charged or amphiphilic molecules, produced by both Gram positive and Gram-negative bacteria. Gram positive Lactic Acid Bacteria produce the most studied and diverse peptides, classified into antibiotics and Cass II peptides. These peptides have antibacterial, immunomodulatory, and antibacterial resistance properties, acting by forming pores in cell membranes and causing cell death. Bacteria secrete peptides as a defence strategy to protect their environment and kill other bacteria. Despite their benefits, there are challenges in clinical use of these peptides. Antimicrobial peptides are produced through ribosomal translation of mRNA or non-ribosomal nonpeptide synthesis, with no ribosomal peptide synthesis mainly occurring in bacteria. They can be produced in vitro through four chemical methods: industrial microorganism culture, genetically modified organisms, enzymatic hydrolysis of proteins, and separation from natural sources. These peptides disrupt the bacterial cell membrane or target within the cytoplasm (Boparai & Sharma, 2019).

AMPs are promising antibiotic alternatives due to their multiple target sites and non-specific action mechanism, reducing resistance development and exhibiting strong anti-biofilm activity against multidrug resistant bacteria. Amps are categorized into four groups: α-helical, β-sheet, extended, and cationic loop. Alpha-helical peptides, which make up 30-50% of all known secondary structures, are the largest group, consisting of 12-40 amino acids and abundant helix stabilizing amino acids like alanine, leucine, and lysine(Yasir et al., 2018).

The rise in antibiotic resistance has led to a growing need for substitute antibiotics. Peptides, with their diverse amino acid sequences and structures, are crucial in understanding biological systems and answering unsolved questions about organisms' natural behaviours. As antibioticresistant strains become more difficult to treat, AMPs have been suggested as potential antimicrobial agents due to their broad-spectrum activity and low resistance potential, making them a promising alternative to current antibiotics. Antimicrobial peptides (AMPs) are positively charged due to their high content of basic amino acids, primarily lysine and arginine, and about half of their hydrophobic amino acids, enabling them to interact with membranes regardless of size or structure (Mejía-Argueta et al., 2020).

Antibiotics have revolutionized medicine, saving millions of lives. However, antibiotic resistance has made treating infectious diseases difficult. Most research has focused on human, agricultural, and veterinary pathogens, but there is growing evidence of non-pathogenic drugresistant microbes in the environmental microbial community. These non-pathogenic microbes could serve as reservoirs for antibiotic-resistant genes, potentially spreading to pathogens through horizontal gene transfer. Despite these threats, the natural environment microbial community resistive has received less attention. Antibiotic resistance is classified into breakpoints like susceptible, intermediate, or resistant. Mangrove extracts have antimicrobial activities, but they are continuously exposed to pollution and deforestation. This paper elucidates the multidrug-resistant nature, biofilm, and community antagonism from several antibiotic-resistant bacteria of mangrove sediment origin (Imchen et al., 2019).

Antibiotics can cause antibiotic resistance, where 90% of bacteria are killed, while the rest adapt and develop strands that allow them to live in the presence of antibiotics. This resistance is caused by regular antibiotic intake or excessive use of the drug, allowing bacteria to mutate their genetic material with resistant properties. Some bacteria learn to neutralize antibiotics or rapidly pump out molecules before acting (Schmidt, 2009).

Antibiotics are used to fight bacteria that cause harm, as they are small molecules produced by bacteria that kill them without inflicting damage on the patient being treated (Pietrangelo, 2017). *E. Coli,* a bacterium that lives in the intestines of the host, causes intestinal infections from contaminated food or water. *Escherichia coli* (*E. coli*) is a commensal and abundant bacterium that colonizes the gastrointestinal tract and mucosal surfaces of various animals, causing severe diseases such as gastroenteritis and extraintestinal infections, affecting global health. *Staphylococcus Aureus,* a bacterium that causes skin infections, can turn deadly if it enters the body's bloodstream and joints. Overall, bacteria can be both helpful and destructive, and antibiotics are used to combat their growth and resistance (Fratamico et al., 2016). *Bacillus,* a large and diverse genus, is known for its diverse medical and industrial applications. Its most beneficial product is antibiotics, primarily peptide antibiotics synthesized through nonribosomal enzymatic processes or ribosomal synthesis of linear peptides. These antibiotics are secreted as secondary metabolites during the late logarithmic or early stationary phase of batch culture growth. The genus's phenotypic diversity and heterogeneity make it a significant taxonomist (Yılmaz et al., 2006). The genus *Pseudomonas* is a diverse bacterial genus with over 60 species found in various sources, including plants, soils, water, and human clinical samples. These obligate aerobic chemoorganotrophs can live on various carbon compounds and are phylogenetically heterogeneous with several subgroups. The genome sequence of four

different *Pseudomonas* species has been recently determined (Jensen et al., 2019). The *Pseudomonas* genus is a diverse bacterial species that inhabits various environments, including plant and animal pathogens. Batrich et al. found *Pseudomonas* species with antibiotic resistance and metal tolerance near Lake Michigan, making it crucial to elucidate the core genome for further applications (Tan et al., 2020).

BACKGROUND

Mangroves are salt-tolerant coastal ecosystems found in tropical and subtropical regions worldwide, providing vital ecological services and habitat for various organisms. These ecosystems are highly productive, supporting diverse flora and fauna, protecting coastlines from erosion and storm damage, and contributing to carbon sequestration and nutrient cycling. Bacteria play essential roles in mangrove ecosystems, such as breaking down organic matter, fixing nitrogen, and mediating biogeochemical processes. Common bacterial groups found in mangroves include Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria, which contribute to the health and functioning of mangrove ecosystems by breaking down organic matter, fixing nitrogen, and mediating biogeochemical processes.

Understanding the bacterial diversity and ecological functions within mangrove ecosystems is crucial for conservation efforts and sustainable management practices, as these habitats face threats like habitat loss, pollution, and climate change. Mangrove bacteria-derived antibiotics offer promising applications in various fields, including medicine and environmental protection. In medicine, these antibiotics treat bacterial infections, including those resistant to conventional antibiotics. Compounds produced by mangrove bacteria, such as streptomycin and erythromycin, have played crucial roles in combating diseases and saving lives. The discovery of novel antimicrobial peptides and secondary metabolites from Proteobacteria and Firmicutes holds potential for developing new drugs to address emerging pathogens and antibiotic-resistant strains. In addition to healthcare, mangrove bacteria-derived antibiotics can also be used in food preservation, agriculture, and bioremediation efforts. By harnessing the antibiotic capabilities of mangrove bacteria, we can address pressing global challenges in healthcare, agriculture, and environmental conservation, leading to sustainable solutions and improved human well-being.

Aim and Objectives

The study aims to explore the antimicrobial properties of mangrove bacteria against human pathogens, with the aim of identifying new compounds that could be utilized in the creation of new antimicrobial drugs or treatments.

Isolation of bacteria from water and soil sediments from mangrove sample.

Characterisation of bacterial isolates.

Screening of bacterial isolates for antimicrobial activities.

Studies on antibiotics peptides.

Hypothesis/ Research Questions

- Mangrove bacteria exhibit antimicrobial properties against a diverse range of human pathogens due to their unique ecological niche and evolutionary adaptations.

  Can bioactive compounds isolated from mangrove bacteria effectively inhibit the growth and virulence of multidrug-resistant human pathogens?

  What are the potential clinical applications of mangrove bacteria-derived antimicrobial agents, and how do they address current challenges in infectious disease treatment and management?

Scope

- Identification of Mangrove Bacterial Strains: Investigate and characterize diverse bacterial species isolated from mangrove environments, focusing on their potential antimicrobial properties.
- Screening for Antimicrobial Activity: Conduct screening assays to evaluate the antimicrobial efficacy of mangrove bacteria against a broad spectrum of human pathogens, including bacteria, fungi, and viruses.
- Bioactive Compound Identification: Isolate and identify bioactive compounds produced by mangrove bacteria responsible for inhibiting the growth of human pathogens.
- Clinical Applications: Explore the feasibility of incorporating mangrove bacteriaderived antimicrobial agents into pharmaceuticals, medical devices, or topical formulations for the treatment of infectious diseases.

CHAPTER 2 LITERATURE REVIEW

Infectious diseases cause 14 million deaths annually, with bacterial infections being a major threat. Antibiotics are the only solution, but the increasing failure of chemotherapy and antibiotic resistance has led researchers to screen plants for their antimicrobial activity. Therefore, there is an urgent need to discover new antimicrobials for new and re-emerging bacterial diseases. Mangroves are rich in bioactive compounds with therapeutic significance, used in traditional medicine for treating human, animal, and plant pathogens. They are also excellent sources of antiviral compounds. This study investigates the antibacterial activity of *Sonneratia alba, Rhizophora mucronata*, and *Exoecaria agallocha* leaves from Chorao island, Goa, collected during the premonsoon season. The phytochemical analysis of mangrove leaf extracts revealed a variety of constituents, including saponins, glycosides, tannins, flavonoids, phenol, and volatile oils. Some metabolites were specific to a species and soluble in a specific solvent. The ethanol extract of *S. alba*, R. *mucronata*, and *E. agallocha* contained saponins, tannins, phenols, glycosides, and volatile oils, while tannins and phenols were present in the aqueous extract (Ansari et al., 2012).

The excessive use of commercial antimicrobial drugs has led to drug resistance in human pathogenic microorganisms, hypersensitivity, immune-suppression, and allergic reactions. Scientists are now seeking new, effective antimicrobial agents to replace current regimens. Mangroves, known for their biologically active antiviral, antibacterial, and antifungal compounds, are being studied for their potential in developing natural antibiotics and discovering alternative treatments for infectious diseases. The study aimed to evaluate the antimicrobial activity of *S. alba* extracts against six human pathogenic microbes, focusing on the use of ripe fruits for intestinal parasite expulsion and half-ripe fruits for cough treatment. The study tested the antimicrobial properties of *S. alba* extracts with various solvents, finding that methanol and ethyl acetate extracts showed variable inhibition activity against tested strains. The study suggests that *S. alba* extracts, rich in tannins, may have potential therapeutic properties for treating *E. coli,* S. *aureus*, and *B. cereus, providing* a potential alternative to traditional antimicrobial drugs (Saad et al., 2012).

Antibiotic-resistant bacteria pose a significant global healthcare issue in the 21stcentury, requiring long-term, effective treatments. Diagnostic costs are high and treatment difficult. Effective strategies for treating and preventing emerging infections are crucial. Novel antibiotics are attractive against bacterial pathogens, with actinomycete species being the most important saprophytic bacteria. These bacteria produce diverse metabolites, making them crucial for discovering new antimicrobial agents. Mangrove environments, found in tropical

and subtropical regions, offer potential for discovering novel bioactive compounds. Actinomycetes isolated from mangrove soils and plants in China and India have shown antibacterial, antifungal, anti-tumour, and protein tyrosine phosphate 1B inhibitory activity, while *Streptomyces sp*. no I-1 from India's Valapattanam mangrove ecosystem showed potential antimicrobial activity. This study focuses on isolating marine-derived actinomycetes from mangrove sediments, a crucial source of new natural products, highlighting the productivity of mangrove ecosystems (Sangkanu et al., 2017).

The ongoing battle against various diseases necessitates urgent efforts to accelerate drug discovery. Indonesian biodiversity offers abundant natural resources, including mangroves, which can be used as potential plant medicines along the entire coastline of Indonesia. This study evaluated the potential of mangrove extracts as antibacterial agents using 16 samples from eight mangrove species. Four solvents (water, ethanol, ethyl acetate, and hexane) were used in maceration, producing 64 extracts. The antibacterial screening was conducted using five bacterial strains using the disk diffusion method.37 extracts of mangroves showed antibacterial potential, with the highest and lowest inhibition indexes being 0.0283 and 1.8983 respectively. The highest was recorded for *Bruguiera gymnorrhiza* root's ethyl acetate extract against *Escherichia coli*, while the second highest was recorded for *Avicennia marina* leaf's water extract against *Staphylococcus aureus*. Most extracts contained saponin and tannin, indicating their potential as antibacterial agents (Audah,2020).

Mangroves, found in saline coastal habitats in tropical and subtropical regions, are of great ecological and socioeconomic significance. The Mandovi-Zuari estuarine complex in Goa, India, is home to various species of mangrove plants. Mangroves have been utilized as folklore medicine since ancient times to combat various bacterial and fungal infections. The study collected three mangrove plant species, *Acanthus illicifolius*, *Ceriops tagal*, and *Sonneretia caseolaris*, from different stations along the Mandovi estuary. The solvent extracts were used to evaluate their antimicrobial activity against human pathogenic bacteria, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*. The results suggest further characterization of active compounds from mangrove plants, potentially used to control drugresistant pathogenic bacteria (Lotlikar & Samant,2015).

The mangrove environment is a valuable source for discovering novel microbiota with potential pharmaceutical science applications. Marine-derived fungi produce secondary metabolites with excellent biomedical applications, potentially treating human and fish diseases. These bioactive compounds have excellent biomedical applications compared to terrestrial sources. The study isolated and tested rhizosphere fungi for their antimicrobial

properties. A potent strain was chosen and grown on a mass scale. Secondary metabolites were extracted, and the crude extract was examined for antimicrobial and antioxidant properties. The *Trichoderma* isolated from mangrove sediment was found to suppress human pathogen growth and possess significant antioxidant properties (Narendran & Kathiresan, 2016).

The study investigates the production of antibiotics from soil microbes of the medicinal plant *Curcuma longa* (Turmeric). Soil samples from Akola City, Western Vidharbh region of Maharashtra were collected. Bacterial cultures were collected using serial dilution and spread plate techniques. Out of 16 cultures, only three were characterized as potent isolates, active against *E. coli*, *P. aeruginosa*, and *S. aureus*. The study identified three cultures of bacteria, *Bacillus megatherium*, *Pseudomonas fluroscenes*, and *Globicitella sulfidifaciens*, using Bergey's manual of systemic bacteriology. The bacterial crude extract of *Bacillus megatherium*, *Pseudomonas fluorescence*, and *Globicitella sulfidifaciens* was found to be more or *Curcuma longa*, a natural antimicrobial source, is less effective against most tested pathogenic strains, making it an alternative to conventional medicines. The best activity was shown by the rhizospheric isolates of *Pseudomonas fluorescens* against *E. coli*, *S. aureus*, and *P. aeroginosa* (Mandale et al., 2017).

Skin disease is the fourth most common disease, with increasing cases and the use of antibiotics for treatment. However, overuse can increase resistance to these pathogens, necessitating urgent study of novel antibiotic compounds against multi-drug resistant (MDR) skin pathogens. The study explores the antimicrobial diversity of 115 bacterial strains associated with three marine nudibranch species from Jepara coastal waters, North Java, in August 2020, and their potential antipathogenic compounds against pathogens *Cutibacterium acnes*, *Staphylococcus aureus*, *Candida albicans*, and *Malassezia furfur*. A study revealed that 24 bacterial isolates (20.87%) demonstrated antimicrobial activity against selected pathogens, including *Bacillus*, from 11 *Firmicutes*, Proteobacteria, and Actinobacteria genera. None of the strains had type-1 polyketide synthases, but one had type II polyketide synthases and five had non-ribosomal peptide synthetase genes. The highest antibacterial strains were found in *G. atromarginata* at 62.5%. The study reveals that nudibranch species harbour diverse bacterial communities, suggesting that many nudibranch-associated bacteria have potential for broad-spectrum antibiotic advancement (Sabdono et al., 2022).

Rhizospheric bacteria with antagonistic effects are a good source for antibiotic production, which are naturally bactericidal or bacteriostatic. Thirty-five rhizospheric bacteria were isolated from soil samples and tested using various methods, including agar well diffusion,

streak agar, disc diffusion, and biochemical tests. *Streptobacillus sp. JRR34* strain, chosen for its primary antagonistic activity, demonstrated strong inhibitory activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The ethyl crude extract of this rhizobacteria, which exhibits strong antagonistic activity against various pathogenic bacteria, could be a valuable source of novel antibiotics (Prasad et al., 2021).

Microorganisms like *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar typhi*, and *Candida albicans* cause wound infections and diseases like diarrhoea and typhoid. Therefore, screening plants that may affect these organisms is crucial for effective medicinal plant use against these pathogens. The investigation aims to assess its antimicrobial activity against potential wound, dental causa, and other pathogens, aiming to identify active principles responsible for its efficacy. The study investigates eight human pathogens, including *P. polyphylla*, and their antimicrobial activity. It presents their susceptibility profile against known antibiotics, with the zone of inhibition in mm and minimum inhibition in mg/ml (Sewa Nakhuru, 2016).

The mangrove ecosystem, a coastal wetland, is home to diverse flora, fauna, and microorganisms. These microorganisms play crucial roles in the ecosystem, such as providing nutrition, decomposing, and producing biotechnology products. This study aims to screen and asses the diversity of cellulolytic bacteria within the mangrove ecosystem. The study investigated cellulolytic bacteria in soil and sediment from the mangrove ecosystem. Three sampling locations were selected, and potential bacteria-producing cellulase were screened using bacteria growth on Carboxy Methyl Cellulose (CMC) agar medium. The bacteria were cultured for 48 hours at 37°C and characterized by morphological and physiological characteristics. The results were adjusted according to Bergey's Manual of Determinative Bacteriology. Six isolates had similar characteristics to *Bacillus*, *Cellulomonas*, *Lactobacillus*, and *Micrococcus*. The cellulolytic activity index showed three isolates as MS06, MS03, and MS08 (Batubara et al., 2022).

The study aimed to evaluate the antimicrobial properties of green tea leaves' extract against skin-associated microorganisms. The extract was tested against Gram positive, Gram negative bacteria, and fungus including *Propionibacterium acne*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* in five solvents: methanol, ethanol, distilled water, chloroform, and petroleum ether. The methanolic extract showed the highest zone of inhibition against *Propionibacterium acne* and *Staphylococcus aureus*, while the ethanolic extract was most effective against *Candida albicans*. It was discovered that the

extracts were more effective against fungus and Gram-positive bacteria than Gram negative bacteria. Comparative studies with antibiotics revealed the methanolic extract had the highest antimicrobial activity. It concluded that *Camellia sinensis* leaves exhibit antibacterial and antifungal properties, making them a potential alternative drug due to their lower side-effects on human skin compared to current therapeutic agents (Sharma & Pundir, 2019).

Drug resistance microbial infection is a significant concern in disease control and treatment. To combat this, a study was conducted to analyse *Bacillus* species as potential sources of antibiotics with antimicrobial activity against resistant strains. Soil samples from various locations were processed to identify *Bacillus* spp. Gram positive inhibitors made up the majority of the isolates, with 53.86% showing broad spectrum activity. *Bacillus* isolates showed effective inhibitory effect against MRSA compared to other test strains. A total of 41 isolates were obtained from the soil processing and screened for antibiotic production. Out of these, 31.70% were screened as antibiotic producers and demonstrated antimicrobial activity against various test organisms selected. The remaining 28 isolates (68.29%) did not exhibit any antimicrobial activity. The study highlights the importance of preventing MDR emergence and developing new drugs to combat this growing threat (Dangol., et al 2019).

The study screened the antimicrobial activities of three *Pleurotus* species, *P. florida*, *P. ostreatus*, and *P. sajor-caju*, against human pathogenic bacteria. The extracts of *mycelia*, pileus, and stipe were prepared using aqueous, hot water, ethanolic, and methanolic methods. The stipe extracts of all three species were effective in inhibiting the growth of these bacteria, with *P. sajor-caju* being the most effective. However, the hot water, ethanolic, and methanolic extracts of pileus were most effective in terms of exhibiting maximum zone of inhibition. *Pseudomonas* species showed the highest zone of inhibition (10.58 mm), followed by *Bacillus* (7.84 mm), and *Streptococcus* (8.44 mm). The minimum average zone of inhibition was recorded against *Proteus* (7.27 mm) and *E. coli* (8.11 mm) (Jarial R.S., et al 2020).

The increasing rate of antimicrobial resistance has led to the search for novel antibacterial compounds to combat infectious diseases. Mangrove ecosystems offer untapped sources of biological active compounds for future antibacterial medicine. The study assessed the antibacterial properties of crude extracts from four prominent mangrove plants in the Ngurah Rai Mangrove Forest: *Rhizophora mucronata, Avicennia marina, Rhizophora apiculata,* and

Sonneratia alba. The roots of these plants were extracted using methanol, chloroform, and nhexane, and tested against two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus* mutans) and two Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) by disc diffusion assay. Results showed that some extracts displayed antibacterial activities against Gram positive strains, but none against Gram negative strains. Only *R. apiculata* exhibited antibacterial properties against both Gram positive indicator strains. A study by Seepana et al. (2016) also showed that methanol, ethanol, and hexane extracts of *R. apiculata* roots were active against *Salmonella typhi* and *S. aureus*, but no activity against *E. coli* (Wijaya & Indraningrat, 2021).

The demand for new antibiotics is increasing due to the rapid spread of antibiotic-resistant pathogens causing life-threatening infections. Scientists are exploring new biologically active antimicrobials with novel mechanisms of action and wide therapeutic potentials. A study was conducted to evaluate the antimicrobial potentials of *Punica granatum* leaf extract against standard and drug-resistant bacteria and *Candida albicans* using agar well diffusion assay and broth dilution methods. The active compounds of these plants were extracted using four different solvents (acetone, methanol, chloroform, and water). The leaf crude extracts showed various degrees of antimicrobial activities, with the maximum mean inhibition zones reaching 22.6 ± 5.50 mm in diameter with methanol leaf extracts against methicillin-resistant *Staphylococcus aureus. Punica granatum* showed superior potential with minimum inhibitory concentrations between 6.25% and 12% for all its extracts against both standard and drugresistant *Streptococcus pneumonia.* Synergetic effects were obtained against methicillinresistant *Staphylococcus aureus* with the combined effects of chloroform extracts of *Punica granatum* with amoxicillin and vancomycin standard antibiotics (Alemu et al., 2017).

CHAPTER 3
METHODOLOGY

Materials and Methods

Collection of Sample

Mangroves soil and water sample were collected from Ribandar, Goa (**Lat - 15.515781° Lat – 15.3988646°)**. Soil and water were collected using a sterile spatula. The soil samples were collected in sterile plastic bags and stored at 4°C till further processing and water sample was collected in the bottle.

Coordinates: (15.504313°) (73.878697°)

Fig 3.1: Sampling site at Diwar Ferry Terminal, Piedade, Goltim-Navelim VP, Goa

Coordinates :(15.398646°) (73.873331°)

Fig 3.2: Sampling sites at Chorao, Goa

Viable count and Isolation of cultures

The bacteria isolation was performed using spread plate method. The sediment sample was weighed as much as 1 gm and suspended in 9 ml of Sterile normal saline (0.85% NaCl) as 10°. Next 0.5 ml of suspension was taken and put into 4.5 ml of test tube containing saline so that dilution factor of 10^{-1}.was obtained. After that, a dilution of 10^{-2} to 10^{-7} were made. Then spread plate 0.1 ml of each dilution of 10^{-5}, 10^{-6}, and 10^{-7} into the NA media plate using sterile spreader. Kept for incubation at room temperature for 24 hours until a colony was grown on the NA plates. For water sample take 1 ml of water sample and add it into 9 ml of sterile saline mix properly and follow the same steps as followed for sediment sample. Isolates were subculture in the tubes for further analysis.

Characterization of bacterial isolates (Bergey's Manual of Determinative Bacteriology)

The bacterial isolates characterization was performed through the observation of microscopic morphological characters and physiological character test using biochemical tests. The microscopic observation was conducted by observing the shape, size, colour of the cells through gram staining. Characterization of microorganisms such as bacteria can be classified based on biochemical reactions. Bacterial isolates can be identified by several biochemical reactions test namely catalase test, citrate test, Methyl Red Voges Proskauer (MR-VP), oxidase test, nitrate test etc.

Gram Staining

A heat-fixed smear was flooded with crystal violet for 1 minute, rinsed, then treated with Gram's iodine for 1 minute and rinsed again. Decolourizer was applied for 3-5 seconds, followed by immediate water rinse. Finally, safranin counterstain was applied for 30-45 seconds, rinsed, and dried. Gram-positive bacteria appear purple due to retained crystal violet, while gram-negative appear pink due to safranin counterstain, observable under high magnification

Spore forming bacteria

Make a smear on clean grease free slide and place the slide over a beaker of steaming water. Cover the slide with blotting paper or tissue with malachite green and let the slide steam for 35 minutes. After that remove the blotting paper or tissue paper and rinse the slide with water to remove any pieces of loose paper and dry the slide. Counterstain with safranin for 1 minute,

then wash with water dry the slide and mount under the microscope and view with oil immersion lens.

Methyl Red test

Bacterial culture was inoculated in specific broth medium (e.g., MR-VP broth). Incubate the tubes at 37℃ temperature for 48 hours. Perform MR Test by adding few drops of methyl Red indicator solution was added to the culture. Observe colour change. Positive results are indicated by the medium turning red, indicating a pH below 4.4, suggesting the presence of acidic fermentation products. Negative results are indicated when the medium remains yellow, indicating a pH above 4.4, suggesting the absence of significant acidic fermentation products.

Voges-Proskauer (VP) test

Bacterial culture was inoculated in specific broth medium (e.g., MR-VP broth). Incubate the tubes at 37°C for 48 hours. Perform VP test, after incubation the reagents were added in correct order, Creatine solution is used to enhances the colour reaction. Alpha-naphthol solution reacts with acetoin to form an intermediate product. Potassium hydroxide (KOH) creates an alkaline environment for the final colour reaction. Observe Colour Change. Positive results indicate the medium turning pink or red, indicating the presence of acetoin. Negative results indicate the medium remains yellow (or light brown), indicating the absence of acetoin.

Citrate test

Bacterial culture was inoculated in medium containing citrate as the sole carbon source. Incubate the tubes at temperature 37°C for 48 hours. pH indicator showed colour change was observed in the medium as pH indicator like common indicators include bromothymol blue or phenol red. Positive test results were indicated if the bacteria utilized citrate, they will produce alkaline by-products, raising the pH and causing the indicator to change colour (e.g., blue for bromothymol blue). Negative test results were indicated if the bacteria cannot utilize citrate, the pH will remain unchanged or become slightly acidic, resulting in no significant colour change in the indicator

Catalase test

Nutrient agar slant-grown pure culture was heavily incubated for 18-24 hours and then 1.0 ml of 3% H_2O_2 was applied. Tubes were placed in dark background to see bubbles form immediately. Positive reactions result in immediate effervescence (bubble formation). A

catalase-negative reaction occurs when no bubbles form due to the absence of the enzyme to hydrolyse hydrogen peroxide.

Oxidase test

Use a loop and pick a well-isolated colony from a fresh (18- to 24hour culture) bacterial plate and rub onto the oxidase disc. Observe for colour changes. Microorganisms are considered oxidase positive when their colour changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the colour changes to purple within 60 to 90 seconds. Microorganisms are considered oxidase negative if the colour remains unchanged or it their reaction time exceeds 2 minutes.

Nitrate reduction test

Bacterial suspension was prepared by inoculating the culture in the nitrate broth. Tubes were incubated at 37^0 c for 48 hours. Look for N_2 gas before adding the reagent. Then add 6-8 drops of nitrate reagent (Salfanilic acid) or 6-8 drops of (alpha – naphthylamine). Observe for colour change if no colour change adds zinc powder and observe for at least 3 minutes for red colour to develop.

Glucose test

The bacterial isolates were inoculated in the Nutrient broth tubes containing 1% of glucose and 1 ml of phenol red, incubated the tubes at RT for 24 hrs and observed for colour change from red to yellow and also the gas production in Durham's tube.

Starch hydrolysing bacteria

The bacterial isolates were streaked on the NA plate containing 0.5% starch powder, incubate the plates at RT for 24 hrs and observe for zone of clearance by adding iodine solution

Antibiotic Sensitivity Test (Dhanraj, 2012)

A small colony of *E. coli, Pseudomonas, Bacillus, S. aureus* was inoculated into the four different flasks containing nutrient broth (NB) of 5 ml using a sterile nichrome loop under sterile condition. The flask incubated at 37° for 24 hours. The turbidity in the flask indicates growth of culture. Then 8 nutrient agar (NA) media plates were taken 2 for each culture. Plates were label properly. Under sterile condition Spread plate 0.1ml of each culture into their label

plates and kept in fridge for 5 mins. After 5 mins made divisions on the plates to place the antibiotic discs (Penicillin(P), Gentamicin (GEN), Vancomycin (VA), Erythromycin(E), Streptomycin(S), Methicillin (MET), Tetracycline (TE), Chloramphenicol (C) Ampicillin (AMP) Polymyxin (PB) Tobramycin (TOB) Trimethoprim (TR). Placed the antibiotic disc in their respective mark with using sterile forceps. Kept the plates for incubation at 37° for 24 hours. The agar plates were removed and zones of inhibition in each agar plates were observed and measured by ruler.

Antimicrobial Assay (Jeganathan et al., 2013)

Isolated colonies were used from the subculture plates than it was inoculated into the test tubes containing nutrient broth of 5ml by using nichrome loop under sterile condition. Then tubes were kept for incubation at room temperature for 24 hours turbidity indicated culture grown. Grown culture were transferred into labelled small centrifuge tubes than the tubes containing culture was centrifuged at 8000 rpm for 10 mins. Pathogens like *E. coli*, *Pseudomonas*, *Bacillus*, *S. aureus* was inoculated into the four different flasks containing nutrient broth (NB) of 5 ml using a sterile nichrome loop under sterile condition. The flask incubated at 37° for 24 hours. The turbidity in the flask indicates growth of culture. Next nutrient agar plates were prepared and Under sterile condition Spread plate 0.1ml of each culture into their label plates and kept in fridge for 5 mins. Mark on the plates the isolates number to place the sterile discs. After that dipped the sterile discs into the different isolate's supernatant using sterile forceps and kept it on their respective marking on the plate. Incubate the plates at room temperature for 24 hours and check for zone of inhibition.

Antibiotic Peptide Extraction (Rai et al., 2016)

Selected the 5 cultures which are showing largest zone of inhibition from the (1.4.5 Antimicrobial Assay). Cultures were grown in five different flask containing nutrient broth and kept for incubation at room temperature for 24 hours. After incubation the culture were transferred into the 50 ml centrifuge tubes and it was centrifuged at 8000 rpm for 10 mins after centrifugation 70% of ammonium sulphate were added in all the centrifuged tubes and kept in the fridge. Next day again centrifuged the tubes at 8000 rpm for 10 min and collected the pellet along with the precipitate by dissolving it with 2 ml of distilled water. Pathogens like *E. coli*, *Pseudomonas*, *Bacillus*, *S. aureus* was inoculated into the four different flasks containing nutrient broth (NB) of 5 ml using a sterile nichrome loop under sterile condition. The flask incubated at 37° for 24 hours. The turbidity in the flask indicates growth of culture. Next nutrient agar plates were prepared and Under sterile condition Spread plate 0.1ml of each

culture into their label plates and kept in fridge for 5 mins. Mark on the plates the isolates number to place the sterile discs. After that dipped the sterile discs into the different isolate's supernatant using sterile forceps and kept it on their respective marking on the plate. Incubate the plates at room temperature for 24 hours and check for zone of inhibition.

CHAPTER 4 RESULTS

Bacterial isolation

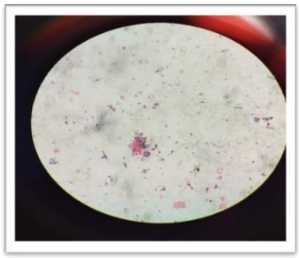
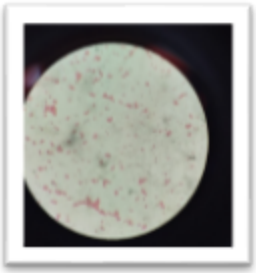
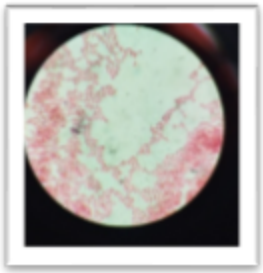
Thirty-four morphological distinct colonies were from mangroves (water and sediment) samples were isolated on sterile nutrient agar plates. These isolates were then purified by spot inoculating on NA plates and were given culture codes. These isolates were characterized based on their morphology and Gram characteristics.

Characterization of bacterial isolates

Colony Characteristics of isolates R1, R2 & R3 were done by checking shape, size, surface, colour, opacity, elevation, margin, time, temperature, gram character and microscopic image. The colony characteristics observed include circular shape, smooth texture, creamy white colour etc. These details provide valuable insights into the morphology and growth pattern of the microbial colony under investigation.

Table 4.1: Colony Characteristics of isolates R1, R2 & R3 from mangrove sample shown in table 4.1.

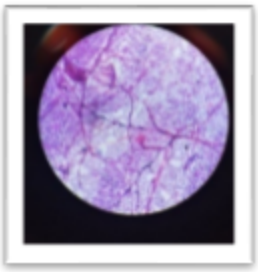
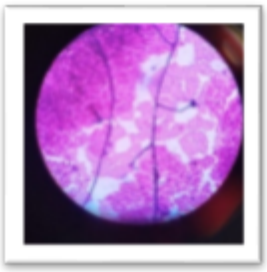
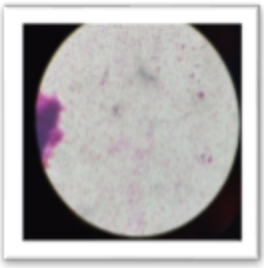
Isolates	R1	R2	R3
Shape	Circular	Circular	Irregular
Size	Medium	Medium	Medium
Surface	Smooth	Smooth	Smooth
Colour	Green	Yellow	Creamy White
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	lobate	Wavy	Wavy
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram Character	Gram positive cocci	Gram negative cocci	Gram negative cocci

Image			

Colony Characteristics of isolates R4, R5 and R6 were done by checking shape, size, surface, colour, opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.2: Colony Characteristics of isolates R4, R5 & R6 from mangrove sample shown in table 4.2.

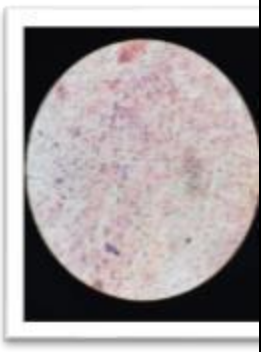
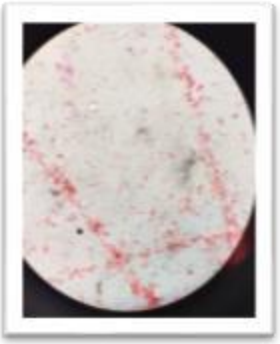
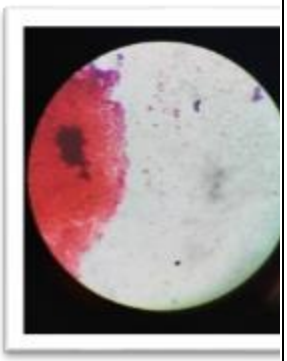
Isolates	R4	R5	R6
Shape	Circular	Circular	Circular
Size	Medium	Small	Small
Surface	Smooth	Smooth	Smooth
Colour	Pinkish	Creamy White	Yellow
Opacity	Opaque	Irregular	Irregular
Elevation	Flat	Flat	Flat
Margin	lobate	Wavy	Wavy
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive cocci	Gram positive cocci	Gram positive cocci

Image			

Colony Characteristics

of isolates R7, R8 and R9 were done by checking shape, size, surface, colour, opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.3: Colony Characteristics of isolates R7, R8 & R9 from mangrove sample shown in table 4.3.

Isolates	**R7**	**R8**	**R9**
Shape	Circular	Circular	Circular
Size	Small	Small	Small
Surface	Smooth	Smooth	Smooth
Colour	Creamy white	Yellow	Pinkish white
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Wavy	Wavy	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive rods	Gram negative cocci	Gram positive cocci
Image			

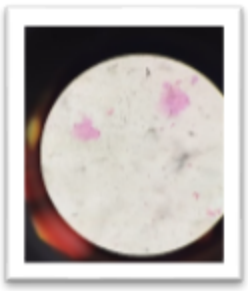
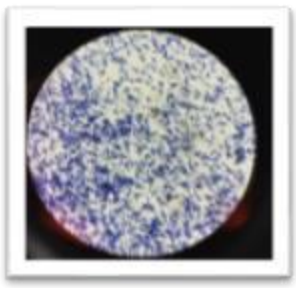
Colony Characteristics

of isolates R10, R11 and R12 were done by checking shape, size, surface, colour, opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.4: Colony Characteristics of isolates R10, R11 & R12 from mangrove sample shown in table 4.4.

Isolates	**R10**	**R11**	**R12**
Shape	Wavy	Wavy	Circular
Size	Small	Small	Small
Surface	Smooth	Smooth	Smooth
Colour	Pinkish white	Yellow	Creamy white
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Lobate	Lobate	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram negative long rod	Gram negative cocci	Gram positive rod

Colony Characteristics

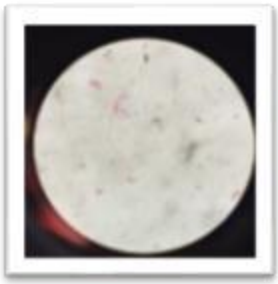
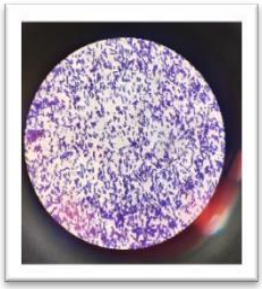
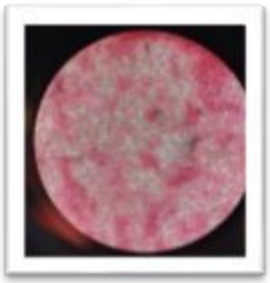
Image			

of isolates R13, R14 and R15 were done by checking shape, size, surface, colour, opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.5: Colony Characteristics of isolates R13, R14 & R15 from mangrove sample shown in table 4.5

Isolates	**R13**	**R14**	**R15**
Shape	Circular	Circular	Circular
Size	Small	Small	Small
Surface	Smooth	Smooth	Smooth
Colour	Yellow	Creamy white	Yellow
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Lobate	Lobate	Even
Time	24hrs	24hrs	24hrs

Colony Characteristics

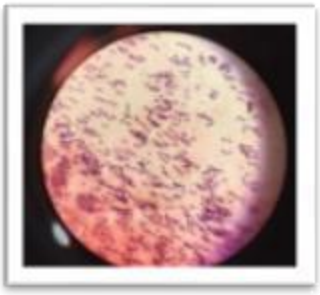
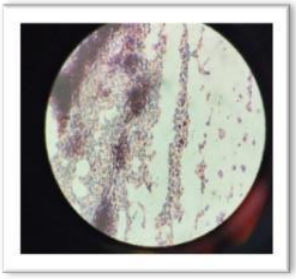
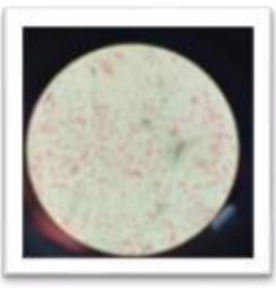
Temperature	RT	RT	RT
Gram character	Gram negative cocci	Gram negative rods	Gram negative cocci
Image			

Colony Characteristics surface, colour

of isolates R16, R17 and R19 were done by checking shape, size,

, opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.6: Colony Characteristics of isolates R16, R17 & R18 from mangrove sample shown in table 4.6

Isolates	**R16**	**R17**	**R18**
Shape	Circular	Circular	Circular
Size	Small	Small	Small
Surface	Smooth	Smooth	Smooth
Colour	White	Creamy	Yellow
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Lobate	Lobate	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive cocci	Gram positive rods	Gram negative cocci
Image			

Colony Characteristics surface, colour

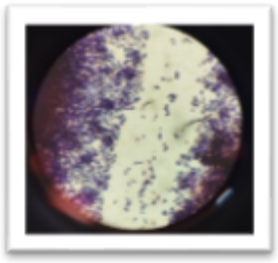
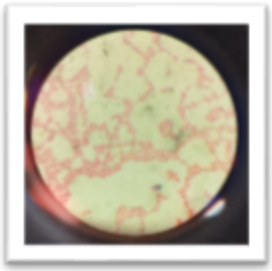
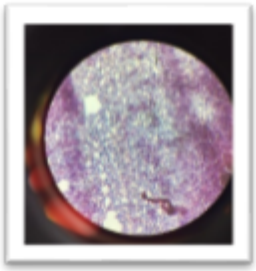
of isolates R19, R20 and R21 were done by checking shape, size, , opacity, elevation, margin, time, temperature, gram character and microscopic image**.**

Table 4.7: Colony Characteristics of isolates R19, R20 & R21 from mangrove sample shown in table 4.7.

Isolates	**R19**	**R20**	**R21**
Shape	Circular	Filamentous	Circular
Size	Small	Medium	Medium
Surface	Smooth	Rough	Smooth
Colour	Creamy White	White	Yellow
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Lobate	Filamentous	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive rods	Gram negative cocci	Gram negative cocci and rods

Colony Characteristics

surface, colour

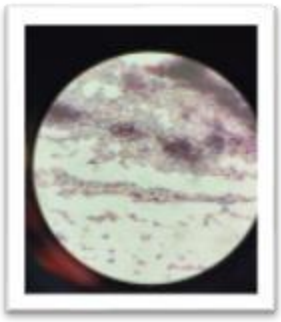
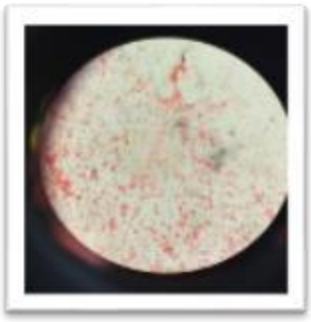
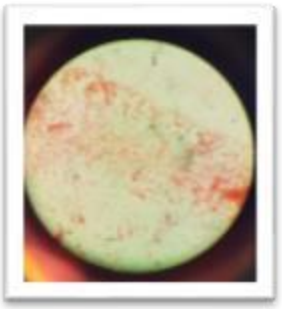
Image			

of isolates R22, R23 and R24 were done by checking shape, size, , opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table 4.8: Colony Characteristics of isolates R22, R23 & R24 from mangrove sample shown in table 4.8

Isolates	**R22**	**R23**	**R24**
Shape	Circular	Circular	Circular
Size	Medium	Medium	Medium
Surface	Smooth	Smooth	Smooth
Colour	Orange	Creamy White	Creamy white
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Even	Even	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT

Colony Characteristics surface, colour

Gram character	Gram positive rods	Gram negative rods	Gram negative cocci
Image			

Colony Characteristics

colour

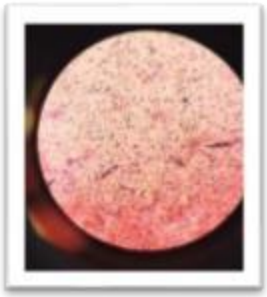
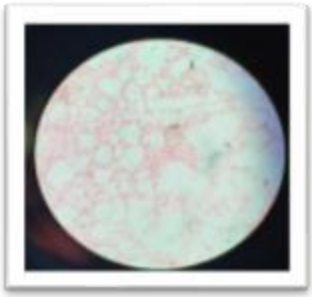
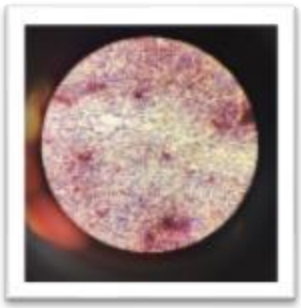
of isolates R25, 26 and R27 were done by checking shape, size, surface, , opacity, elevation, margin, time, temperature, gram character and microscopic image.

Table: 4.9 Colony Characteristics of isolates R25, 26 and R27 from mangrove sample shown in table 4.9

Isolates	**R25**	**R26**	**R27**
Shape	Circular	Circular	Circular
Size	Medium	Medium	Medium
Surface	Smooth	Smooth	Smooth
Colour	White	Yellow	Creamy white
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Even	Even	Even
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive rods	Gram positive cocci	Gram negative cocci

Colony Characteristics

colour

Image			

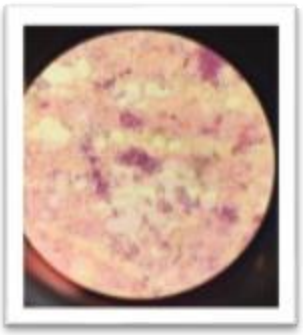
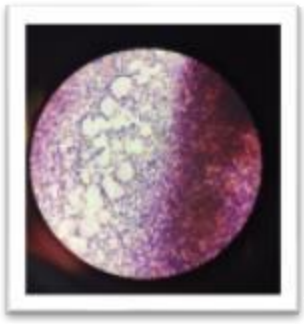
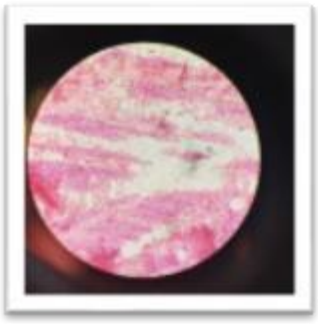
of isolates R28, 29 and R30 were done by checking shape, size, surface, , opacity, elevation, margin, time, temperature, gram character and microscopic image

Table 4.10: Colony Characteristics of isolates R28, R29 & R30 from mangrove sample shown in table 4.10.

Isolates	**R28**	**R29**	**R30**
Shape	Circular	Circular	Filamentous
Size	Medium	Medium	Medium
Surface	Smooth	Smooth	Smooth
Colour	Pinkish	Yellow	Creamy white
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Margin	Even	Even	Filamentous
Time	24hrs	24hrs	24hrs
Temperature	RT	RT	RT
Gram character	Gram positive rods	Gram positive cocci	Gram positive rods

Colony Characteristics

colour

Image			

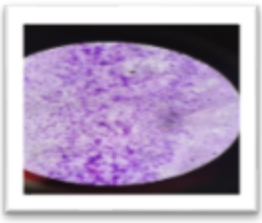
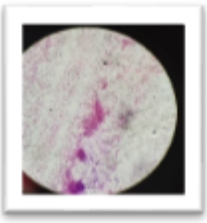
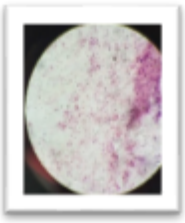
of isolates R28, 29 and R30 were done by checking shape, size, surface, , opacity, elevation, margin, time, temperature, gram character and microscopic image

Table 4.11: Colony Characteristics of isolates R31, R32, R33 & R34 from mangrove sample shown in table 4.11

Isolates	**R31**	**R32**	**R33**	**R34**
Shape	Circular	Circular	Filamentous	Filamentous
Size	Medium	Medium	Medium	Medium
Surface	Smooth	Smooth	Smooth	Smooth
Colour	Yellow	Creamy white	White	Creamy white
Opacity	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat	Flat
Margin	Even	Even	Filamentous	Filamentous
Time	24hrs	24hrs	24hrs	24hrs
Temperature	RT	RT	RT	RT

Colony Characteristics

colour

Gram character	Gram negative cocci	Gram negative cocci	Gram positive cocci	Gram negative cocci
Image				

Biochemical identification

Biochemical identification for Gram positive rods

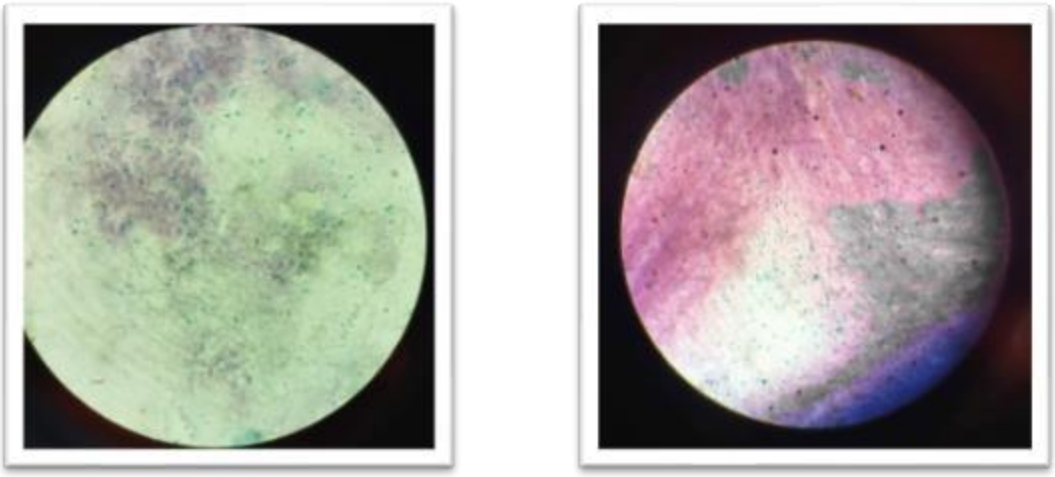
Spore Forming Bacteria

Out of 4 isolates only two isolate shows green colouration shown in figure Fig 3: Bacterial isolates A (R17) and B (R21) showing spore formation of bacterial cell which means it producing spores.

Bacterial isolates identified as Gram-positive rods, A (R17) and B (R21) showed spore formation.

Table 4.12: Isolates selected for spore formation

Culture number	**Spore formation**
R17	**Spore formed**
R21	**Spore formed**
R22	No Spore formed
R30	No Spore formed



A **B**

Fig 4.3: Bacterial isolates A (R17) and B (R21) showing spore formation.

Biochemical identification for Gram negative rods and negative cocci

Nitrate reduction test

Out of 7 bacterial isolates 3 bacterial isolates showed positive result for Nitrate reduction test shown in fig 5 and table 4.13.

The culture R14, R27 & R23 has the ability to produce nitrate reductase enzyme.

Table 4.13: Observation for the Nitrate Reduction Test in the table 4.13 given below.

Isolates	Nitrate reduction
R3	-
R24	-
R11	-
R14	+
R15	-
R27	+
R23	+

Key: + Presence of nitrate reducers; - Absence of nitrate reducers

Fig 4.4: Nitrate Reduction Test Control

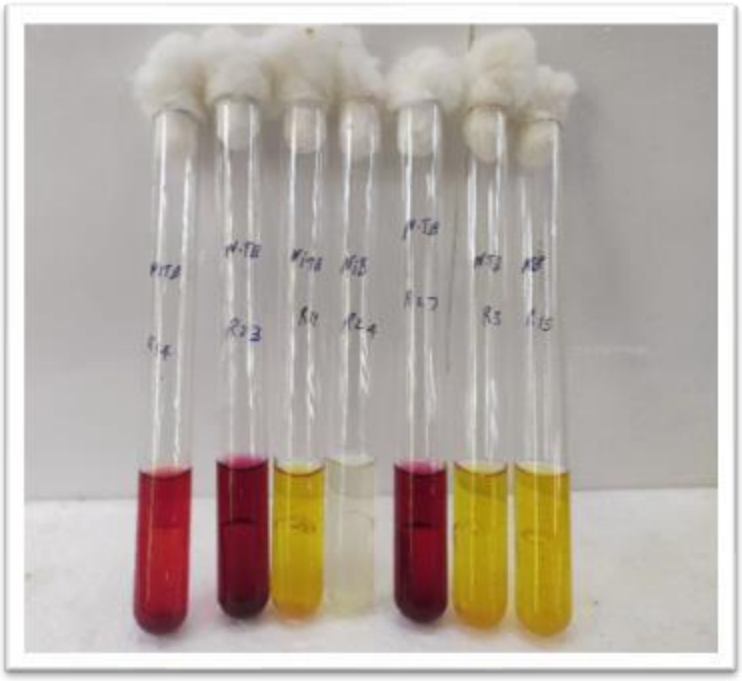


Fig 4.5: Nitrate Reduction Test (In order – R14, R23, R11, R24, R27, R3 and R15).

Biochemical identification for Gram positive rods

Hugh Leifson Test

Out of 4 bacterial isolates isolate R30 has shown negative result can be seen in fig 4.6 & 4.7. A positive result indicated growth and utilization of glucose under an aerobic condition (R17, R21 and R22), while the a negative would indicate no growth.

Table 4.14: Result and interpretation of the Hugh Leifson test.

Isolates	Aerobic (+) or Anaerobic (-)	
	With oil	Without oil
R21	+	+
R17	+	+
R30	-	-
R22	+	+

Key: + Aerobic bacteria; - Anaerobic bacteria

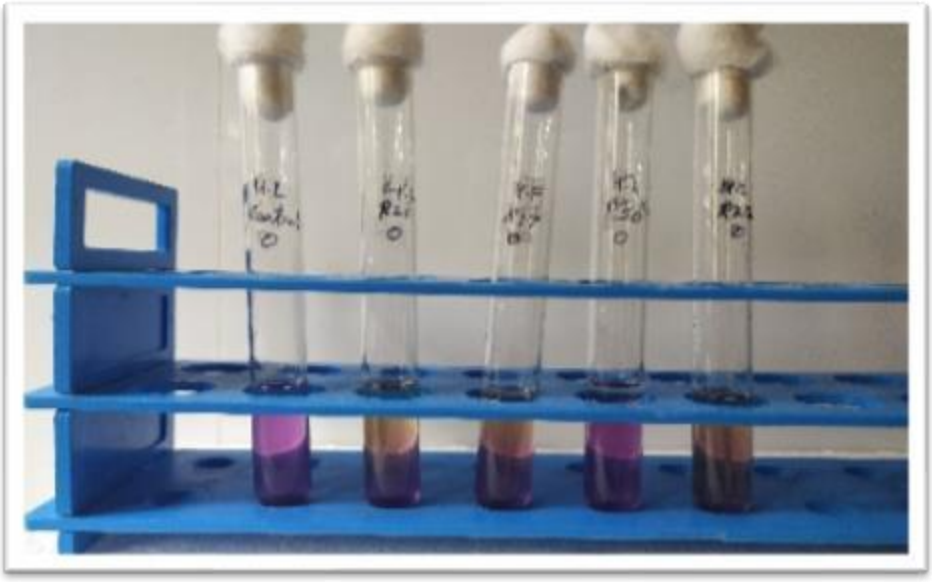


Fig 4.6: Colour of medium with inoculation of culture in tube with Paraffin Oil.

(In order – Control, R21, R17, R30, and R22).

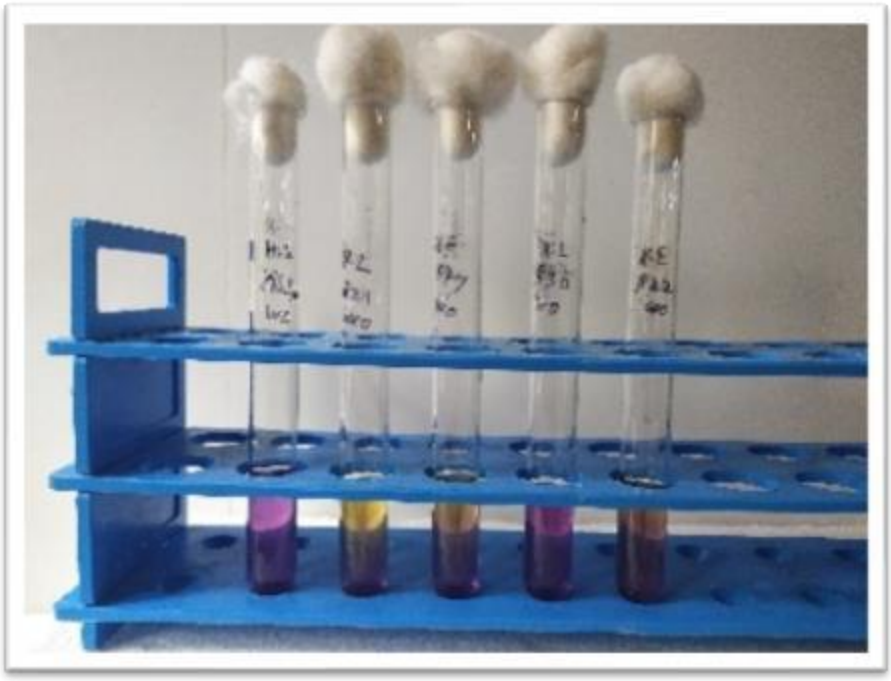


Fig 4.7: Colour of medium with inoculation of culture in tube without Paraffin Oil (In order – control, R21, R17, R30 and R22).

Fig 4.8: Hugh Leifson test was carried out by using isolates R21, R17, R30, R22 and Using control

Biochemical identification for Gram positive rods

and negative cocci.

Starch hydrolysing bacteria

Out of 9 isolates only one isolates shows starch hydrosis by showing Of clearance around the colonies shown in figure 4.9.

A positive result is indicated by the formation of a clear halo around the colonies and development of dark blue to purple color in surrounding medium after the addition of gram's iodine. Bacterial isolates showing no zone of clearance around the colonies except for the isolates R22 and R30.

Negative result indicated by no halo around the colonies.

Table 4.15: Bacterial isolates showing starch hydrolysis test.

Isolates number	Zone of clearance
R17	-
R 3	-
R11	-
R15	-
R24	-
R27	-
R30	+
R22	+
R21	-

Key: + Zone of clearance; - No zone of clearance

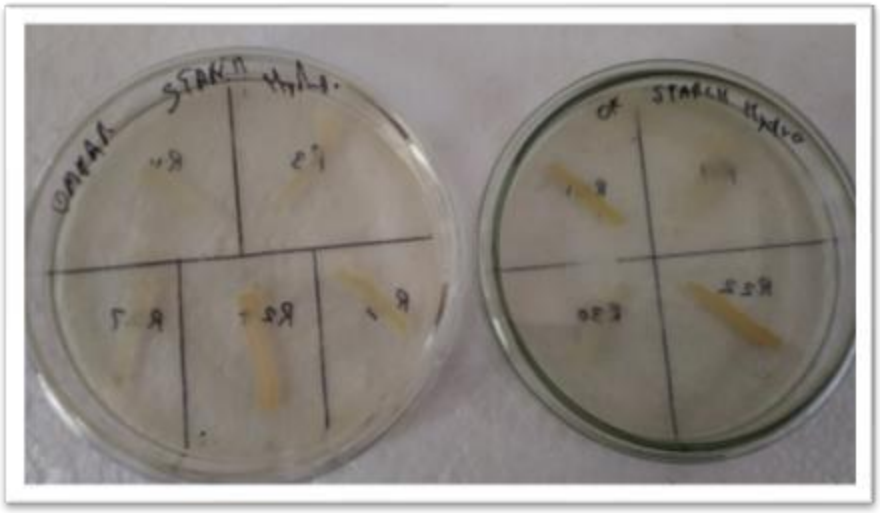


Fig 4.9 : Bacterial isolates showing no zone of clearance around the colonies except for the isolates R22 and R30.

Biochemical identification for Gram negative, positive rods and positive cocci.

Glucose test

Out of 10 bacterial isolates Bacterial isolates showing glucose fermentation shown in figure 4.10

Isolates R30, R14, R23, R21 and R28 showing Glucose fermentation with acid production and with gas formation while R6, R22, R17, R1, and R26 shows Glucose fermentation with acid production and no gas formation.

Table 4.16: Bacterial isolates fermenting glucose

Isolates number	Glucose fermentation
R30	++
R6	+
R22	+
R17	+
R14	+ +
R23	++
R1	+
R21	++
R26	+
R29	++

Key: + + Glucose fermentation with acid production and with gas formation; + Glucose fermentation with acid production and no gas formation

Biochemical identification for Gram positive rods

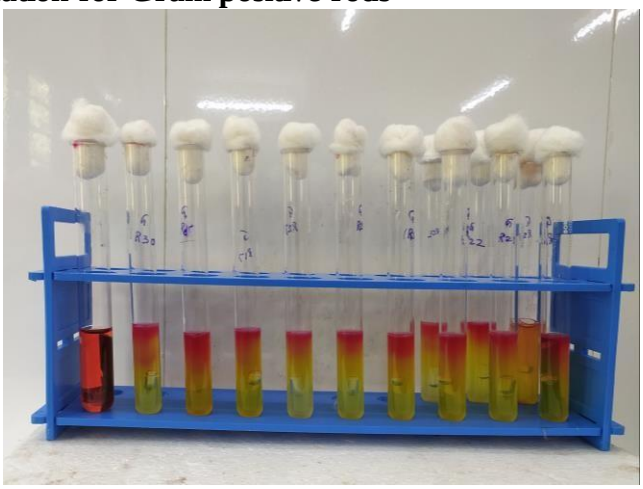


Fig 4.10: Bacterial isolates showing glucose fermentation (In order: Control, R30, R6, R17, R23, R1, R21, R26 and R29.

Catalase test

All the 13 bacterial isolates were showing bubble formation after adding hydrogen peroxide; indicating catalase positive as shown in figure 4.11,4.12,4.13,4.14,4.15,4.16 and 4.17.

All the bacterial isolates are showing the bubble formation.

Table 4.17: Bacterial isolates showing catalase test.

Culture number	Citrate producers
R1	+
R6	+
R30	+
R22	+
R21	+
R26	+
R11	+
R15	+
R27	+
R29	+
R3	+

R17	+
R24	+

Key: + Catalase production

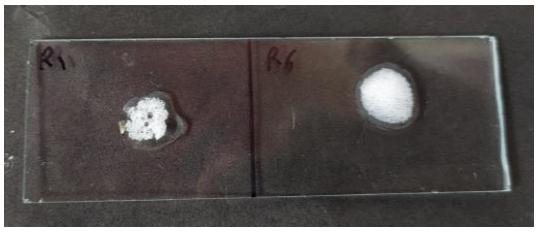


Fig: 4.11 Isolate R1 and R6

Fig: 4.12 Isolate R22 and R30

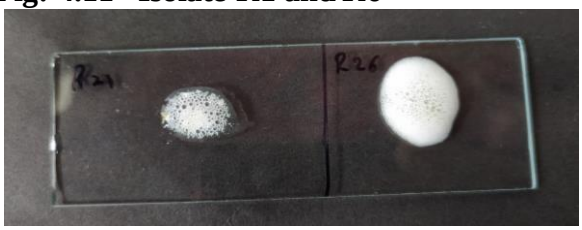


Fig 4.13: Isolate R29 and R26

Fig 4.14: Isolate R11 and R15

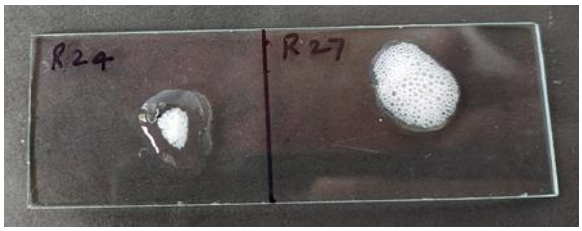


Fig 4.15: Isolate R24 and R27

Fig 4.16: Isolate R17 and 21

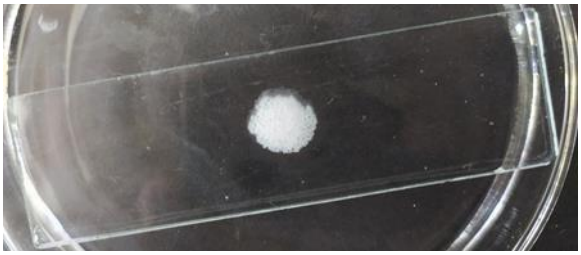


Fig 4.17: Isolate R3

Biochemical identification for Gram positive rods

Biochemical identification for Gram negative cocci.

Citrate test

Out of 5 bacterial isolates only 4 isolates were identified as citrate producers. Showing colour change from Green to blue. Shown in figure 4.18.

Positive reaction: Growth with colour change from green to intense blue along the slants (R3, R15, R27 and R11).

Negative reaction: No growth and no colour change; Slant remains green(R24).

Table 4.18: Bacterial isolates showing citrate utilization.

Culture number	Citrate producers
R3	**+**
R15	**+**
R27	**+**
R11	**+**
R24	**-**

Key: + Citrate utilization; - No Citrate utilization

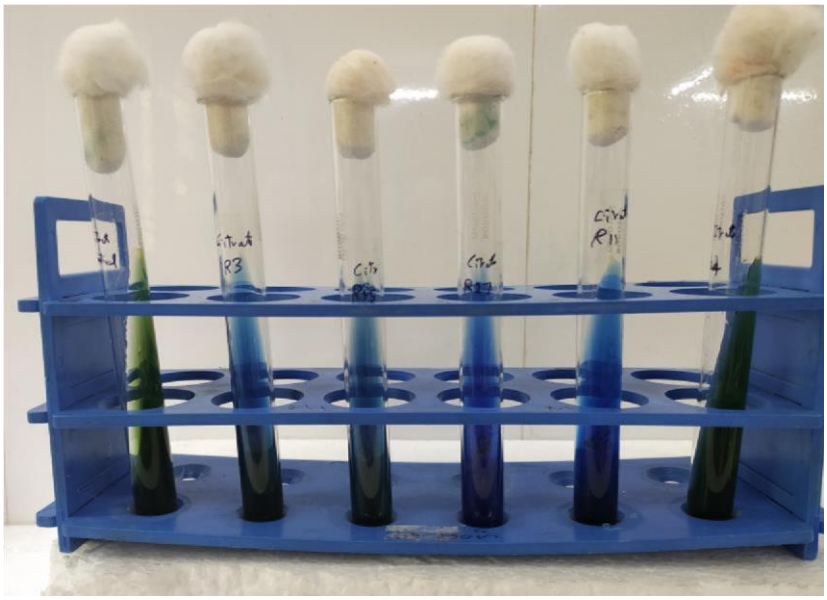


Fig: 4.18 Isolates showing citrate activity (In order: control, R3, R15, R27, R11 and R24.
Biochemical identification for Gram negative cocci.

MR-VP test

Out of 5 bacterial isolates 2 bacterial isolates has found to be positive result as shown in figure 4.19.

A) Methyl Red (MR) test

Positive reaction: A distinct red colour (Isolates R15 and R11).

Negative reaction: A yellow colour (Isolates R3, R27, and R24).

B) Voges Proskauer (VP) test

A positive result is indicated by a lack of pink-red colour over the surface of the medium.

A negative result is indicated by a lack of pink- red colour over the surface of the medium or the formation of the copper colour (R15, R11, R3, R27 and R24).

Table 4.19: Bacterial isolates showing Voges Proskauer test

Isolate Number	VP test
R15	**+**
R11	**+**
R3	**-**
R27	**-**
R24	**-**

Table 4.20: Bacterial isolates showing Methyl red test

Isolate Number	VP test
R15	**-**
R11	**-**
R3	**-**
R27	**-**
R24	**-**

Key: + Red ring formed; - No red ring formed

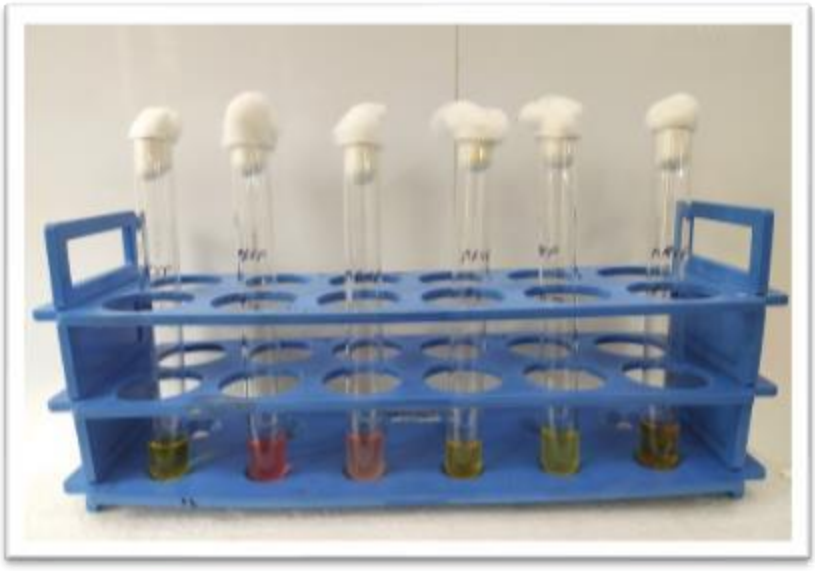


Fig: 4.19 Bacterial isolates showing Methyl red test (In order – Control, R15, R11, R3, R27 and R24.

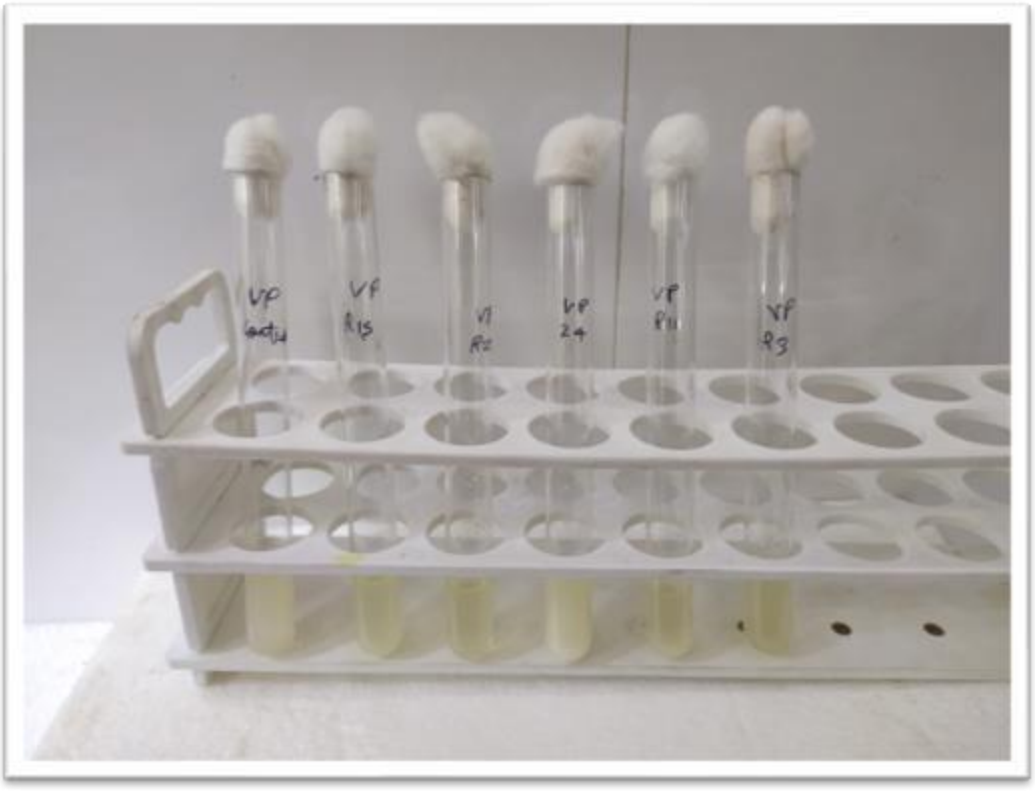


Fig 4.20: Bacterial isolates showing Voges Proskauer test (In order – Control, R15, R11, R3, R27 and R24.

<u>Biochemical identification for Gram negative rods.</u>

Oxidase producing bacteria

Out of 2 bacterial isolates 1 isolate gives positive results for by showing colour changes to dark purple shown in figure 4.21.

Positive test: Development of purple to deep blue colour within 15- 30 seconds indicates a positive oxidase test (Isolate R14).

Negative test: No development of purple to deep blue colour within 3 minutes (Isolate R23).

Table 4.21: Isolates showing oxidase production

Culture number	Oxidase production
R14	**-**
R23	**+**

Key: - No catalase formation; + Catalase formation

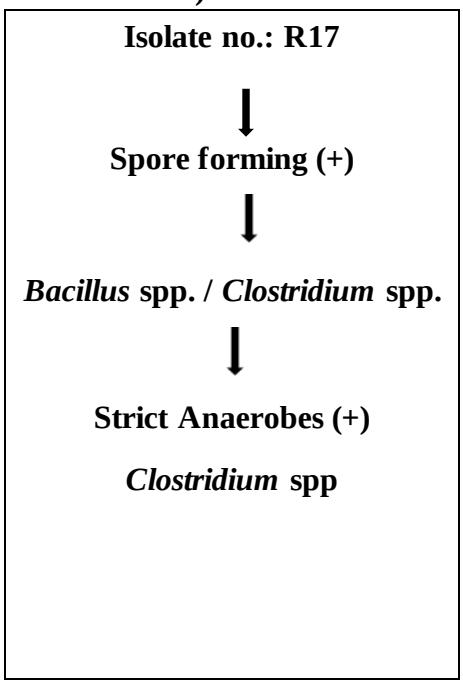
Fig 4.21: Bacterial isolate showing positive result R14(left) and Bacterial isolate showing negative result R23(right).

Identification of bacterial Isolate

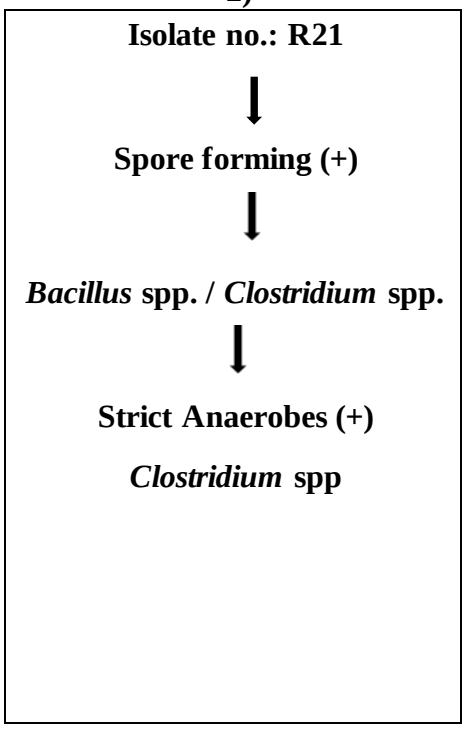
Bacterial isolates were identified using a Bergy's manual.

1) Gram positive rods

1)



2)



3)

Isolate no.: R22

↓

Spore formation (-)

***Corynebacterium* spp.**

***Lactobacillus* spp.**

***Mycobacterium* spp.**

↓

Catalase (+)

***Corynebacterium* spp.**

↓

Starch hydrolysis (+)

Corynebacterium kutsceri

4)

Isolate no.: R30

↓

Spore forming (-)

***Corynebacterium* spp.**

***Lactobacillus* spp.**

***Mycobacterium* spp.**

↓

Catalase (+)

***Corynebacterium* spp.**

Starch hydrolysis (+)

Corynebacterium kutsceri

2) Gram negative rods **1)**

Isolate no.: R14

↓

Oxidase test (+)

Aeromonas, Pseudomonas, Vibrio

↓

Glucose fermentation (+)

***Vibrio* spp., *Aeromonas* spp.**

2)

Isolate no: 11

↓

Oxidase test (-)

Enterobacteriaceae

↓

Lactose fermentation (+)

Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter intermedius, Erwinia carotovora, Erwinia chrysanthemi, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia fonticola, serratia rubidaea

3) Gram positive cocci

1)

Isolate no.: R1

Catalase (+)

Micrococcus spp., Staphylococcus spp.

2)

Isolate no.: R6

Catalase (+)

Micrococcus spp., Staphylococcus spp.

3)

Isolate no.: R21

Catalase (+)

Micrococcus spp., Staphylococcus spp.

4)

Isolate no.: R26

Catalase (+)

Micrococcus spp., Staphylococcus spp.

5)

Isolate no.: R29

↓

Catalase (+)

Micrococcus spp., Staphylococcus spp.

4) Gram negative cocci

Isolates number R3, R11, R15, R24 and R 27 identified as gram-negative cocci, hence it identified as *Neisseria* or *Veillonella* sp

Antibiotic Sensitivity Test

All the pathogens are showing resistance to the Methicillin (MET), Ampicillin (AMP), Trimethoprim (TR).

1: *E. coli* is exhibited sensitivity to Streptomycin (S) with a zone of inhibition 24 mm, moderate sensitivity to Vancomycin (VA) with a zone of inhibition 18 mm and resistance to Methicillin (MET), Ampicillin (AMP), Trimethoprim (TR) with no zone of inhibition.

2: *Bacillus* is exhibited sensitivity to Gentamicin (GEN) with a zone of inhibition 23 mm, moderate sensitivity to Vancomycin (VA) with a zone of inhibition 12 mm and resistance to Methicillin (MET), Ampicillin (AMP), Trimethoprim (TR) with no zone of inhibition

3. *Pseudomonas* is exhibited sensitivity to Streptomycin (S) with a zone of inhibition 19 mm, moderate sensitivity to Tetracycline (TE) with a zone of inhibition 9 mm and resistance to Methicillin (MET), Ampicillin (AMP), Trimethoprim (TR) with no zone of inhibition

4: *S. aureus* is exhibited sensitivity to Chloramphenicol (C) with a zone of inhibition 33 mm, moderate sensitivity to Erythromycin (E) with a zone of inhibition 20 mm and resistance to Methicillin (MET), Ampicillin (AMP), Trimethoprim (TR) with no zone of inhibition

Table 4.22: Antibiotic Sensitivity Test Against human pathogens

Antibiotic disc	**Zone of inhibition in mm**			
	E. coli	***Bacillus***	***Pseudomonas***	***S. aureus***
Penicillin (P)	9 mm	8 mm	-	7 mm
Gentamicin (GEN)	23 mm	20 mm	17 mm	30
Vancomycin (VA)	18 mm	12 mm	17 mm	15 mm
Erythromycin (E)	19 mm	18 mm	-	20 mm
Streptomycin (S)	24 mm	20 mm	19 mm	25 mm
Methicillin (MET)	-	-	-	-

Tetracycline (TE)	18 mm	20 mm	9 mm	21 mm
Chloramphenicol (C)	18 mm	17 mm	2 mm	33 mm
Ampicillin (AMP)	-	-	-	-
Polymyxin (PB)	19 mm	12 mm	-	15 mm
Tobramycin (TOB)	19 mm	17 mm	-	17 mm
Trimethoprim (TR)	-	-	-	-

Fig 4.22: Antibiotic Discs

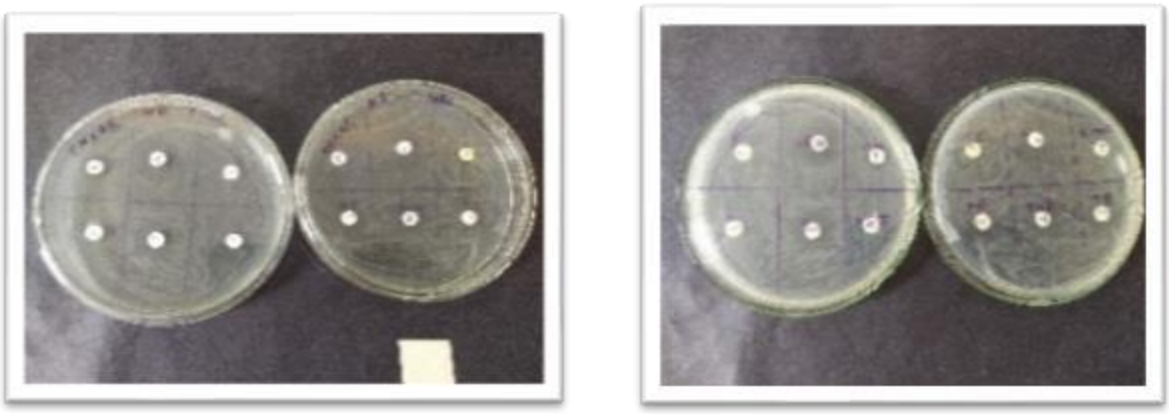


Fig 4.23 : *E.coli* is showing resistance to 7 antibiotics disc (P, E, MET, AMP ,PB, TOB, TR).

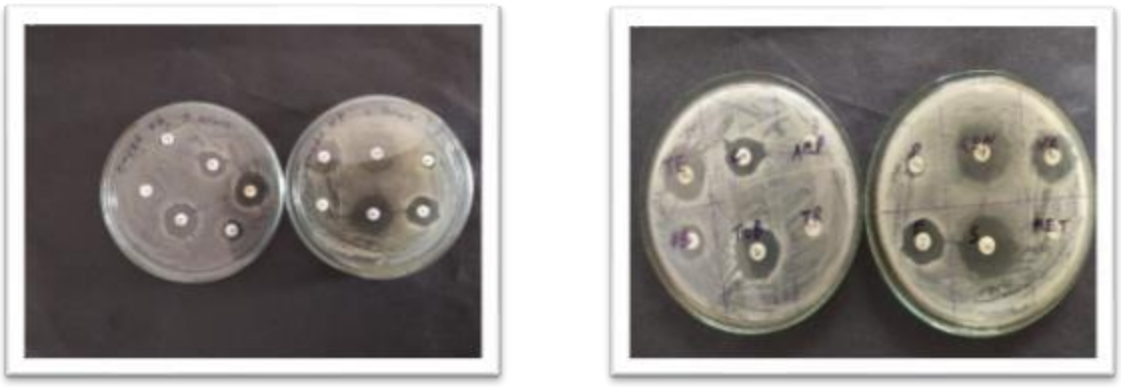


Fig 4.24: *S. aureus* is showing resistance to 2 antibiotics discs (MET AND TR).

Fig 4.25: *Pseudomonas* is showing resistance to 2 antibiotics disc (MET AND TR).

Fig 4.26: *Bacillus* is showing resistance to 3 antibiotics disc (MET, AMP and TR).

Antimicrobial Assay

Among the 27 bacterial isolates R1, R6, R15, R20 and R32 are showing larger zones among the other isolates against host cultures showed in figure 4.25, 4.27, 4.28 and 4.26.

Isolate R15 showed the highest zone of inhibition against the *Bacillus* culture, whereas R15 showed the lowest zone of inhibition against the *S. aureus* culture.

Table 4.23: Zone of inhibition of isolates against human pathogen.

	Zone of inhibition (mm)			
Isolates	*Pseudomonas*	*E. coli*	*Bacillus*	*S. aureus*
R1	11	**17**	9	9
R3	9	9	10	8
R6	**18**	11	**13**	9
R7	12	8	11	8
R8	8	6	9	10
R9	17	6	9	8
R11	10	8	8	9
R12	9	8	7	9
R14	10	12	8	8
R15	08	**16**	**18**	2
R16	08	14	11	9
R17	12	9	7	10
R18	08	9	11	8
R19	11	8	8	8
R20	**11**	**12**	**17**	8
R21	11	11	9	8
R22	11	11	10	10
R23	08	9	10	9
R24	10	8	11	7
R26	09	13	8	11
R27	12	11	9	9
R28	10	9	7	10
R29	07	8	9	11

R30	09	11	7	12
R31	08	8	7	11
R32	06	**14**	**16**	9
R33	09	07	11	8

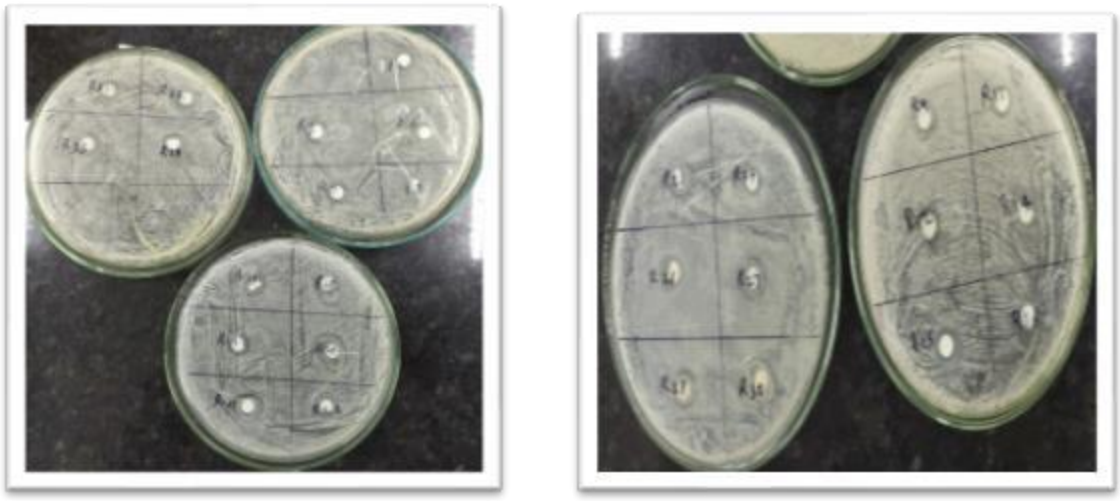


Fig 4.27: Bacterial isolates showing zone of inhibition against *E. coli.*

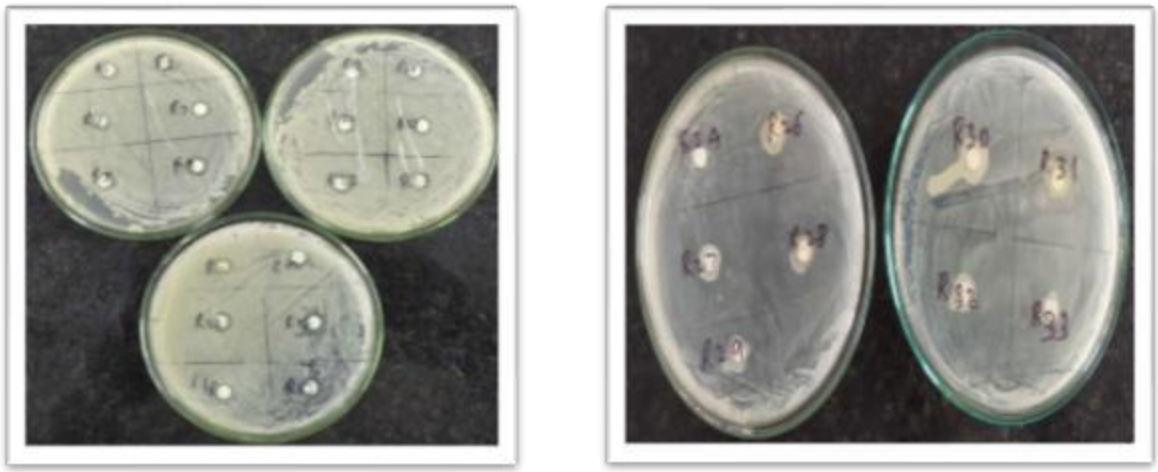


Fig 4.28: Bacterial isolates showing zone of inhibition against *S. aureus.*

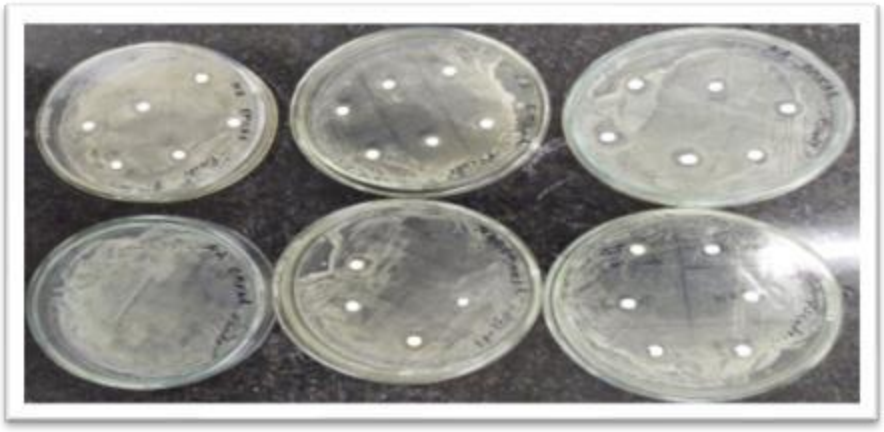
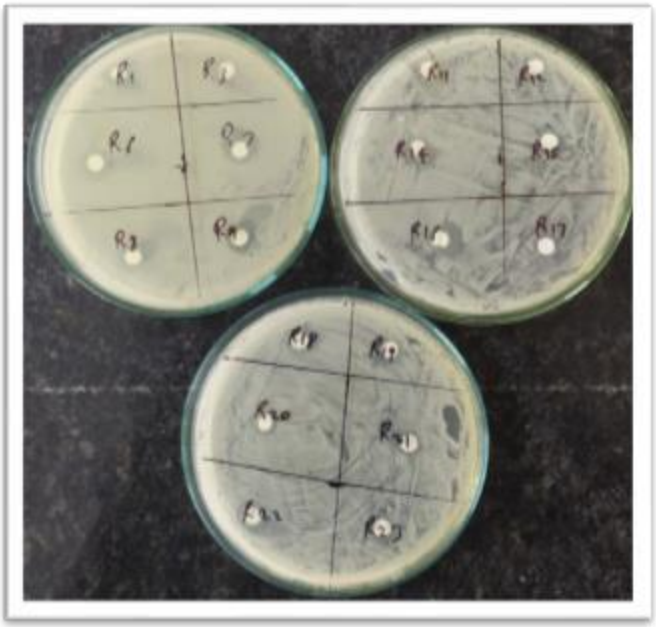


Fig4. 29: Bacterial isolates showing zone of inhibition against *Pseudomonas.*



a)

b)

Fig 4.30: Bacterial isolates showing zone of inhibition against *Bacillus.*

Antibiotic Peptide Extraction (ammonium sulphate precipitation)

Isolates which were giving larger zone of inhibition from the Antimicrobial Assay activity from table 4.22 were chosen to extract antibiotic peptide from those isolates (This is done by adding ammonium sulphate and centrifugation and using precipitate to use against human pathogens). Results shown in figure 4.31, 4.32, 4.33 and 4.34.

Bacterial isolate R20 which shows zone of inhibition against all host culture shows potential implication for antibiotic development or clinical.

Table 4.24: Using antibiotic peptide against human pathogens

Isolates	**Zone of inhibition** against human Pathogens (mm)			
	Pseudomonas	*Bacillus*	*S. aureus*	*E. coli*

R1	-	3	6	13
R6	-	7	6	-
R15	6	6	4	-
R20	7	7	6	8
R32	7	7	5	-

Key: - No zone of inhibition

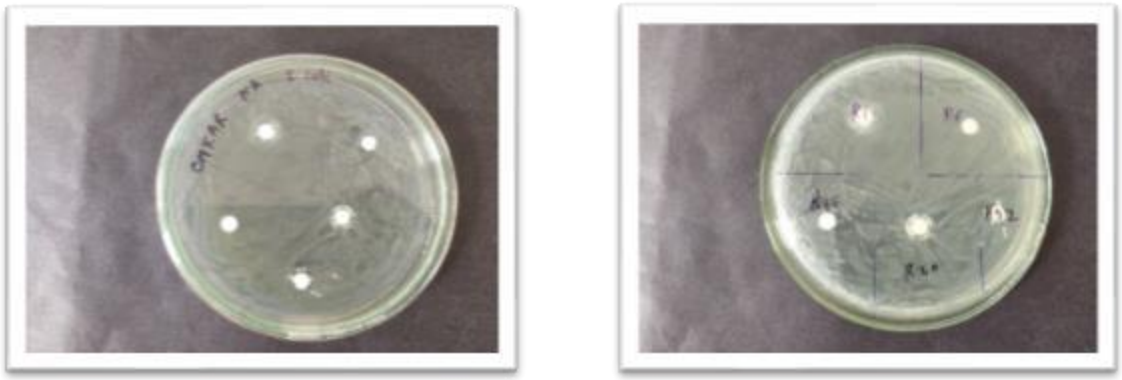


Fig 4.31: Only 2 bacterial isolates R1 and R20 shows zone of inhibition after extracting peptide and using it against *E. coli.*

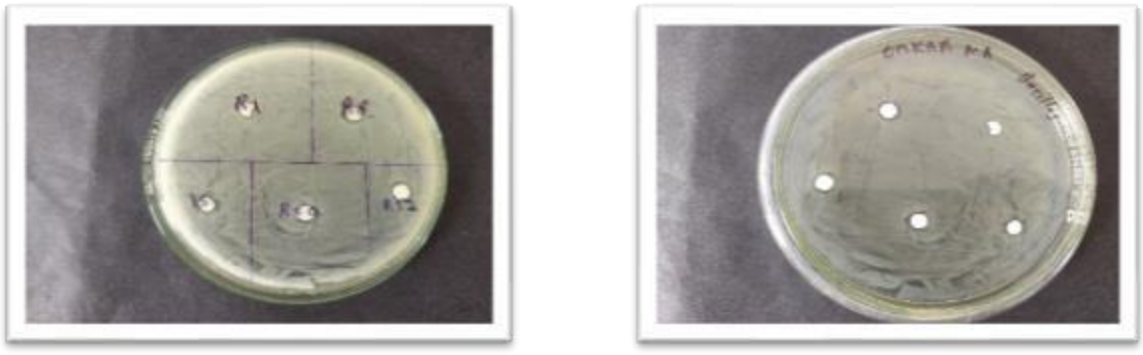


Fig 4.32: All bacterial isolates shows zone of inhibition after extracting peptide and using it against *Bacillus.*

Fig 4.33: Bacterial isolates R15, R20 and R32 shows zone of inhibition after extracting peptide and using it against *Pseudomonas*.

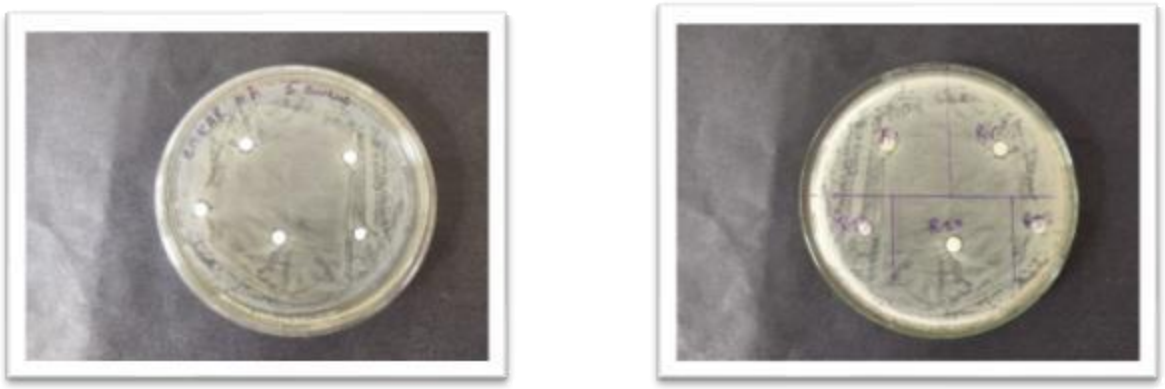


Fig 4.34: All bacterial isolates shows zone of inhibition after extracting peptide and using it against *S. aureus*

CHAPTER 5
DISCUSSION AND CONCLUSIONS

DISCUSSION

Antimicrobial assay was done by using Total 28 bacterial isolates tested against the human pathogens (*E. coli, Pseudomonas*, *Bacillus* and *S. aureus*) by using a disc method where bacterial isolates showed large "zones of inhibition" appearing around the discs indicating that the particular bacterial isolate is effective against the present bacterial strains. Various size of inhibition has been showed in this test against the human pathogens by the bacterial isolates. In their study (Lotlikar & Naik Samant, 2015) the antimicrobial assay was conducted on Mangrove plant extracts using the agar-well diffusion method, specifically the Muller-Hinton agar. It was found that aqueous extracts of mangrove plant species did not show any inhibitory activity against pathogens. The highest inhibitory activity was observed in methanolic extracts of *Acanthus illicifolius* (0.5cm), followed by *Ceriops tagal* (0.5cm). The highest zone of inhibition was observed against E. coli (0.8 cm), followed by *Ceriops tagal* (0.5cm). The highest inhibitory activity against *B. subtilis* (0.7 cm). *S.aureus* (0.8 cm and 1.0 cm).Whereas bacterial isolates R8 show highest (18mm) and R32 show lowest(6mm) zone of inhibition against the *Pseudomona*s culture, isolate R16 highest (14mm) and R8(6mm) lowest zone of inhibition against *E.coli*, Isolate R6 highest(13mm) and R3(6mm) zone of inhibition against *Bacillus* and isolate R30 highest(12mm) and R15(2mm) against *S.aureus*. Both the study highlights the potential of mangrove plants like *Acanthus illicifolius, Ceriops tagal*, and *Sonneretia caseolaris* and also the mangrove associated bacteria in Goa as a source of therapeutic antibacterial compounds.

Antibiotic sensitivity test was carried out using antibiotic disc such Penicillin (P),Gentamicin (GEN), Vancomycin (VA), Erythromycin (E), Streptomycin (S), Methicillin (MET), Tetracycline (TE), Chloramphenicol (C), Ampicillin (AMP), Polymyxin (PB), Tobramycin (TOB), Trimethoprim (TR) against human pathogens such as E.*coli, S.aureus, Bacillus* and *Pseudomonas* by a disc method. *E. coli* exhibits sensitivity to Streptomycin with a 24 mm zone of inhibition, moderate sensitivity to Vancomycin with an 18 mm zone of inhibition, and resistance to Methicillin, Ampicillin and Trimethoprim. *Bacillus* exhibits geneticin sensitivity with a 23mm zone of inhibition, moderate geneticin sensitivity with a 12mm zone of inhibition, and resistance to Methicillin, Ampicillin, and Trimethoprim. *Pseudomonas* has a 19mm zone of inhibition for Streptomycin, 9mm zone for Tetracycline, and no zone of inhibition for Methicillin, Ampicillin, and Trimethoprim. *S. aureus* is exhibited sensitivity to Chloramphenicol (C) with a zone of inhibition 33 mm, moderate sensitivity to Erythromycin (E) with a zone of inhibition 20 mm and resistance to Methicillin (MET), Ampicillin (AMP),

Trimethoprim (TR) with no zone of inhibition. In this study (Akhter et al., 2013) the disc diffusion method was used to measure antimicrobial sensitivity on Mueller-Hinton agar plates for organisms other than *Streptococci,* which required blood agar plates. Different antibiotic discs used in sensitivity tests (quantity/disc) are: IMP: Imipenem (10 µg), AZT: Aztreonam(30 µg), CIP: Ciprofloxacin (5 µg), TE: Tetracycline (30 µg), AMP: Amoxicillin (30 µg), SXT: Cotrimoxazole (25 µg), GM for Gentamycin (120 µg), ATH: Azithromycin (15 µg), NA: Nalidixic acid (30 µg), NI: Nitrofurantoin (300 µg), CFX: Cephalexin (30 µg) .The study reveals that most antibiotic-resistant organisms are becoming increasingly resistant over time. Most isolates showed 50% or higher sensitivity to imipenem, azithromycin, and cephalexin, except for *Staphylococci.* All isolates were highly sensitive to imipenem, even up to 100% by *Staphylococci. E. coli* showed less than 50% sensitivity to 8 antibiotics out of 11, while Klebsiella showed 20-40% sensitivity to 5 antibiotics out of 11. *Pseudomonas* was completely resistant to TE, AMP, NI, CFX, and highly resistant to AZT, CIP, GM, and NA by 20%.

Extraction of antibiotics peptide was carried out by using ammonium sulfate precipitation where disc method was used by the 5 isolates(R1, R6, R15 , R20 and R32) tested against the human pathogens (*E.coli, Pseudomonas, Bacillus and S.aureus*)Where isolate R20 showed the zone of inhibition against the all host cultures (*E.coli, Pseudomonas, Bacillus and S.aureus*). Isolate R1 showed the highest zone of inhibition (13 mm) against the *E. coli.* In their study (Jasir et al., 2004) the antibacterial activity of Cystapep 1 derivative was evaluated using the agar well diffusion method Cystapep 1, a drug, showed antibacterial activity against several Gram-positive bacteria, with minimal inhibitory and bactericidal concentrations of about 16 µg/ml for *Staphylococcus aureus* and *Streptococcus pyogenes*. It was generally susceptible to groups A, B, C, and G *Streptococci, Staphylococci, Pneumococci,* and *Enterococci,* but not Gram-negative bacteria. Cystapep 1, a small, modified amino acid peptide, has a selective antibacterial spectrum that may be advantageous for ecological reasons. Its mode of action may differ from known membrane pore-forming peptides, as it is much smaller and contains extensive modified amino acid residues. Cystapep 1 demonstrated significant activity against MRSA and CNS, indicating a unique mechanism of action compared to most current antibiotics. This indicates that in both the studies Cystapep 1 and Bacterial isolates R20 has potential new sources of antibiotics to combat antibiotic resistance, the ability to target specific pathogens with lower chances of resistance development, and the opportunity for more sustainable and environmentally friendly antibiotic production methods compared to traditional chemical synthesis. Additionally, these peptides may have diverse applications in medicine, agriculture, and biotechnology due to their antimicrobial properties.

Conclusions

- The bacteria isolated from mangrove has shown antibiotic resistance to human pathogens based on the activities done such as Antimicrobial Assay and Antibiotic peptide extraction.

- Total 34 morphologically distinct colonies were isolated on sterile nutrient agar and isolates were characterized based on their morphology and Gram character.

- Bacterial isolates were identified by doing bacterial identification using Bergey's Manual where

  R17 and R21 isolates belonging to *clostridium* spp.

  R30 isolate belonging to *cornybacterium kutsceri.*

  R14 isolate belonging to *vibrio* spp., *Aeromonas* spp.

  R11 isolate belong to *Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter amnigenus Enterobacter intermedius, Erwinia carotovora, Erwinia chrysanthemi, Escherichia coli, Klebsiella oxytoca Klebsiella pneumoniae, Serratia fonticola, Serratia rubidaea.*

  R1, R6, R21, R26 and R29 isolates belonging to *Micrococcus* spp. *Staphylococcus* spp.

  Isolates number R3, R11, R15, R24 and R27 belonging to *Neisseria* or *Veillonella* sp.

- Isolate number R15, R1, R6, R20 and R32 were showing the Highest zone of inhibition against the human pathogens after doing Antimicrobial Assay.

- Isolate Number R20 has shown zone of inhibition against all host culture which shows potential Implications for antibiotic development or clinical use.

- The bacterial isolates have demonstrated concerning antibiotic resistance against human pathogens. Through comprehensive testing methods, including susceptibility assays and resistance profiling, it was observed that the isolate exhibited resistance to multiple antibiotics commonly used in clinical settings. The resistance extended to critical antibiotics vital for treating severe infections, raising alarms about the potential challenges in managing bacterial infections.

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APPENDIX

1) Nutrient Agar

Ingredients	Grams/Litre
Peptone	**5.000**

Sodium chloride	**5.000**
HM peptone B#	**1.500**
Yeast extract	**1.500**
Agar	**15.000**
Final pH (at 25°)	**7.4±0.2**

 # - Equivalent to Beef extract

2) Nutrient Broth

Ingredients	**Grams/Litre**
Peptic digest of animal tissue	**5.000**
Beef extract	**3.000**
Potassium nitrate	**1.000**
Final pH (at 25°C)	**7.0±0.2**

3) Huge Leifson medium

Ingredients	**Grams/Litre**
Peptone	**2.000**
Sodium chloride	**5.000**
Dipotassium phosphate	**0.300**
Glucose	**10.000**
Bromothymol blue	**0.050**
Agar	**2.000**

Final pH (at 25°C)	**6.8±0.2**

4) MR-VP Broth

Ingredients	Grams/Litre
Buffered peptone	**7.000**
Dextrose	**5.000**
Dipotassium phosphate	**5.000**
Final pH (at 25°C)	**6.9±0. 2**

5) Nitrate Broth

Ingredients	Grams/Litre
Peptic digest of animal tissue	**5.000**
Beef extract	**3.000**
Potassium nitrate	**1.000**
Final pH (at 25°C)	**7.0±0.2**

6) Gram's iodine

Ingredients	Grams/Litre
Solution A	
Crystal violet (90% dye content)	**2g**
Ethanol,95%	**20 ml**

Solution B	
Ammonium oxalate	0.8g
Distilled water	80ml

Mix Solution A and B. Store 24hr and filter through coarse filter paper.

7)Methyl red

Ingredients	Grams/Litre
Methyl red	0.200gm
Ethyl alcohol	60.000ml
Distilled water	40.000

8) Phenol red

Ingredients	Grams/Litre
Phenol red sodium salt	0.04gm
Distilled water	100ml

9) Malachite green

Ingredients	Grams/Litre
Malachite green crystals	0.5gm
Distilled water	100ml

Stir until crystal are dissolved, filter to remove residual crystals.