

**SEAGRASS- DERIVED ANTIMICROBIAL PEPTIDES AS
QUORUM SENSING INHIBITORS FOR HUMAN
PATHOGENS AND INDUCE APOPTOSIS OF MCF7
BREAST CANCER CELL LINE**

PROJECT WORK

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

By

**RAJESHWARI K (41300032)
RIHADHA FARHIN M (41300036)**



**SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY -1 UNIVERSITY BY UGC

Accredited with Grade "A++" by NAAC | 12B Status by UGC | Approved by AICTE

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Rajeshwari K** (41300032) and **Rihadha Farhin M** (41300036) who carried out the project entitled "SEAGRASS-DERIVED ANTIMICROBIAL PEPTIDE AS QUORUM SENSING INHIBITORS FOR HUMAN PATHOGEN AND INDUCE APOPTOSIS OF MCF7 BREAST CANCER CELL LINE" under my supervision from January 2024 to April 2024.

Internal Guide

Dr. S. JAYASHREE

25/04/24

External Guide

Dr. D JEYAPRAGASH

HEAD OF THE DEPARTMENT

Dr. V. RAMESH KUMAR

Submitted for Viva-voce Examination held on 27/04/2024

Internal Examiner

External Examiner

DECLARATION

We, Rajeshwari K (41300032) and Rihadha Farhin M (41300036) hereby declare that the Project Report entitled "SEAGRASS-DERIVED ANTIMICROBIAL PEPTIDE AS QUORUM SENSING INHIBITORS FOR HUMAN PATHOGEN AND INDUCE APOPTOSIS OF MCF7 BREAST CANCER CELL LINE" done by us under the guidance of Dr.S.Jayashree, Internal Guide, Assistant professor, Department of Biotechnology and Dr.D.Jeyapragash, External guide at Centre for Ocean Research ,Sathyabama Research Park is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology .

DATE:27.04.2023

PLACE: CHENNAI



SIGNATURE OF THE CANDIDATE

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ABSTRACT

Antimicrobial peptides (AMPs) are promising tool that can be used to combat drug-resistant pathogens. However, the high cost involved in AMP synthesis and their short plasma half-life render their clinical translation a challenge. To address these shortcomings, we report efficient isolation of bioactive AMPs from *Halodule pinifolia*. Moreover, AMPs from seagrass exhibit robust killing activity against one fungal strain and two highly virulent and antibiotic resistant ESKAPE pathogens prevent their biofilm formation and shown potential anticancer activity against *MCF7* cell. Taken together, our experimental data demonstrate the potential use of seagrass based Antimicrobial peptides (AMP) used for pharmaceutical application.

Keywords: Antimicrobial peptides, *Halodule pinifolia*, Anti-fungal, Anti-bacterial.

LIST OF CONTENT

S.no	TITLE	PAGE NO
	ABSTRACT	I
	CONTENT	II
	LIST OF ABBREVIATIONS	IV
	LIST OF TABLES	V
	LIST OF FIGURES	VI
1	INTRODUCTION	1
2	LITERATURE SURVEY	3
3	AIM AND OBJECTIVES	
	3.1 AIM	5
	3.2 OBJECTIVES	5
4	MATERIAL AND METHODS	
	4.1 <i>H. Pinifolia</i> collection and Extract Preparation	6
	4.2 Protein Quantification	6
	4.3 LC-MS ANALYSIS	7
	4.4 AMP Prediction by Bioinformatic analysis	7
	4.5 Antifungal Activity	7
	4.6 Antibacterial Activity	7
	4.7 Determination of Minimum Inhibitory Concentration (MIC)	8
	4.8 Growth Kinetics	9

4.9 Light Microscopic Visualization	9
4.10 Anticancer Activity	
4.10.1 Cell line and culture	10
4.10.2 Reagents	10
4.10.3 In Vitro assay for Anticancer assay(MTT assay)	11
5 RESULTS AND DISCUSSION	
5.1. Protein Concentration Assessment	12
5.2. MS Analysis of the Peptide Sequences Identified in <i>H. pinifolia</i> Extracts	13
5.3. AMP Prediction by Bioinformatic Analysis	14
5.4. Antifungal Activity	14
5.5. Antibacterial Activity	15
5.6. Minimum Inhibitory Concentration	17
5.7. Growth Kinetics	18
5.8. Light Microscopic Visualization	21
5.9. Anticancer Activity	23
6 SUMMARY AND CONCLUSION	29
REFERENCES	31

LIST OF ABBREVIATIONS

ACPs	Anticancer Peptides
AMPs	Antimicrobial peptides
AMR	Antimicrobial Resistance
BSA	Bovine Serum Albumin
C.A	<i>candida albicans</i>
DMSO	Dimethyl sulfoxide
FBS	Fetal Bovine Serum
<i>H. Pinifolia</i>	<i>Halodule Pinifolia</i>
LB	Luria-Bertani
LC-MS	Liquid Chromatography-Mass Spectrometry with Electrospray Ionization
MIC	Minimum Inhibitory Concentration
MTT	Methyl Thiazolyl diphenyl- Tetrazolium bromide
OP	optical density
P.aeruginosa	Pseudomonas aeruginosa
PS	Phospholipid phosphatidylserines
RPM	Revolutions Per Minute

LIST OF TABLE

TABLE NO	CONTENT	Page.NO
5.1	Peptide sequences identified in <i>H. pinifolia</i> extracts using bioinformatic analysis	14
5.2	Cell viability of MCF7 cell line against AMP	24

LIST OF FIGURE

S.NO	FIGURE NAME	P.NO
4.1	Seagrass <i>Halodule pinifolia</i>	6
5.1	Quantification of Antimicrobial peptide of Bradford method	12
5.2	LCMS analysis of AMP from <i>Halodule pinifolia</i>	13
5.3	Zone of inhibition of AMP against <i>C.Albicans</i>	15
5.4	Zone of inhibition of AMP against <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> .	16
5.5	Antimicrobial Peptide Inhibit the biofilm formation of <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>E. faecalis</i>	
5.6	Growth curve of <i>Candida albicans</i> in the presence/absence of AMP	19
5.7	Growth curve of <i>P. aeruginosa</i> in the presence/absence of AMP	19
5.8	Growth curve of <i>K. pneumoniae</i> in the presence/absence of AMP	20
5.9	Effect of methanol extract on biofilm cells and planktonic growth. After 24 h incubation, control and treated <i>C. albicans</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> were photographed under light microscope (40X Magnification). Extract treated groups exposed a reduction in biofilm formation.	22
5.10	Anticancer effect of Sample on <i>MCF7</i> Cell line	24
5.11	Anticancer effect of Sample on <i>MCF7</i> Cell line microscopic image	25

CHAPTER 1

INTRODUCTION

Seagrasses are aquatic plant found in all ocean and continental except the Antarctica. Seagrass are present 72 species approximately around the world, compared to 250000 terrestrial angiosperm. Before 70 million to 100 million years ago single monocotyledons flowering plant are develop from single lineage seagrass with 4 families viz. *Hydrocharitaceae*, *Cymodoceaceae*, *Posidonaceae* and *Zosteraceae* (Orth R J *et al.*, 2006). Seagrass ecosystem provide habitat for small fish, turtle feed of shellfish and mantae as well as waterflow. seagrass are grow in both littoral zone and neritic zone in all the tropical ocean.(Short, F. T *et al.*, 2015). The close ecological relationships between plants and micro-organisms have been acknowledged for more than 150 years as a crucial component of the health and growth of terrestrial vegetation (Seymour, J. R *et al.*, 2018). All land plants growth, health, and ecology are largely shaped by endophytic, rhizospheric, and epiphytic microbes. These microbes also play a significant role in agricultural processes, soil fertility and chemical cycling, vegetation succession, and the biological productivity of terrestrial ecosystems. There are significant mutualistic, commensal, and pathogenic interactions in plant-microbe relationships. A variety of microorganisms can be found living in the leaves of seagrass, such as fungi (Raghukumar, S., & Raghukumar, S *et al.*, 2014). and epiphytic bacteria (Trevathan-Tackett, S. M *et al.*, 2020). In protista (Garcias-Bonet., 2011). Though a thorough knowledge of these interactions within the leaf microbiome is still lacking, it is likely that these epiphytic microbes interact with the seagrass host, algal epiphytes, and each other. Our understanding of bacterial abundances and community composition on seagrass leaf tissues has improved due to recent developments in microscopy, cytometry, and molecular biology (Tasdemir, D. *et al.*, 2024). Seagrass meadows offer essential ecological services, like serving as breeding grounds. Providing food, cover, and habitat for local wildlife, some of which have a high commercial value (Pazzaglia, J *et al.*., 2021). for a number of species. Seagrasses are considered ecosystem engineers because they give epi- and endobenthic organisms intricate three-dimensional structures, prevent erosion by holding onto sediments, and efficiently filter pollutants and nutrients.

carbon dioxide, temperature rise, and acidification. Seagrass accurately describes a class of angiosperms that are particularly suited to flourish in marine environments and estuaries. As therefore, there are 13 genera in this group of plants, and there are about 72 species (Jackson, E. L *et al.*, 2015). The stabilization of sea floor sediment, which inhibits erosion, and the recycling of nutrients are two significant ecosystem services linked to seagrasses. Storm-relation and offers vital habitat for spawning fish and a large number of young marine animals. Seagrasses are a source of food and habitat. They act as the main source of nutrition for manatees and green sea turtles. as well as dugongs, and they sustain a wide range of fish, drawing a variety of predators after them. Numerous coastal communities across the globe, including those in India (Rengasamy R K *et al.*, 2020) have documented the significance of seagrasses Sweden (Folmer E O *et al.*, 2016). Creating a climate refuge by locally buffering mild temperature increases (Crespo, D., *et al.*, 2021). It is crucial to encourage measures to stop the downward trend in such demanding circumstances .Many studies have been conducted on antimicrobial natural products derived from plants in the hopes that they could prevent the spread of the numerous newly discovered antibiotic-resistant pathogens. Plants are the sources production of secondary metabolites and antimicrobial peptides. It has been demonstrated that bacterial densities vary seasonally, with summertime peak abundances corresponding with increased photosynthetic production and modifications in the synthesis of antimicrobial compounds (Wahbeh and Mahasneh *et al.*, 1984). These novel inhibitory agents derived from natural products are anticipated to be safe, effective, and to have minimal negative effects on the environment (Supaphon, P *et al.*, 2011).

CHAPTER 2

LITERATURE SURVEY

Millions of years ago, terrestrial plants gave rise to the class Monocotyledons, which includes seagrasses. Nonetheless, there hasn't been much research done on antimicrobial peptides from seagrasses (Horinouchi, M *et al.*, 2009). Seagrasses are among the ocean's richest and most significant coastal habitats, providing home to a variety of keystone and ecologically significant marine species across all trophic levels. These plants, which belong to the class Monocotyledoneae, are underwater blooming plants that create enormous meadows. They originated on land and returned to the sea millions of years ago, where they now flower and seed. In addition to being a vital component of more complex ecosystems found in marine coastal zones, seagrasses also form an important marine habitat on their own. They support the health of salt marshes, oyster reefs, coral reefs, and mangroves (Unsworth R *et al.*, 2014). Because of their high primary productivity and role in the detrital cycle, seagrasses support a variety of marine food webs, both within their seagrass beds and as wrack that washes ashore (Hemminga M A *et al.*, 2000). They also supply organic carbon and nutrients (N and P) to other ocean regions, including the deep sea, and play a major role in carbon sequestration (Duffy J E *et al.*, 2006). Seagrass ecosystem services are worth US\$34,000 per hectare annually (Dewsbury B M *et al.*, 2016) here recalculated to 2010 dollars), which is more than many terrestrial and marine habitats combined. Millions of people live in coastal communities, mostly in tropical regions, and artisanal fisheries are also dependent on seagrass habitats for their livelihoods. Consequently, these serve as a vital source of food for larger animals related to seagrass. Many commercial, recreational, and subsistence fish and shellfish use seagrasses as a nursery ground for their juvenile and larval stages (Short F *et al.*, 2007). Antimicrobial peptides (AMPs) are the first line of defense against pathogens and are ancient effector molecules of the innate immune defense system of all living things. These cationic, small, amphipathic peptides are thought to be promising due to their selective toxicity, broad antimicrobial spectra, and other advantages over antimicrobial resistance (AMR) and general mechanism of action, which centers on focusing on the primary structural components of the bacterial membrane, decreasing the rate at which microbial resistance develops.

The microorganisms in contrast to traditional antibiotics (Waycott M *et al.*, 2011). Furthermore, a number of AMPs have demonstrated adjuvant or synergistic effects with widely used antibiotics (Bugli F *et al.*, 2022), and they have served as models for the in silico rational design of novel, enhanced, and selective medications. Numerous studies conducted recently have shown that cationic AMPs can be examined as a novel class of anti-tumoral drugs to combat tumor resistance to established chemotherapy. These compounds may be hazardous; they are also known as anticancer peptides (ACPs) their capacity to bind the tumor cells, without having an adverse effect on healthy cells, Phospholipid phosphatidylserines (PS) are found in the plasma membrane's outer leaflet in cells that are cancerous (Wodlej C *et al.*, 2019). Several AMPs and ACPs have been isolated to date from a variety of natural sources, including insects and amphibians and angiosperms. However, it can be challenging to obtain representative quantities of peptides in research.

Seagrasses, a type of marine plant, have drawn a lot of attention lately because they produce secondary metabolites that are thought to have biological significance and may be employed as possible pharmacological substances (Faulkner *et al.*, 2016). In the Mediterranean Sea, the seagrass *Posidonia oceanica* is an endemic plant and is crucial to the marine ecosystem ecological functioning (Duarte C M *et al.*, 1894). Few studies have been reported this seagrass have pharmacological important antimicrobial peptides having potential antitumor activity (Hale J A *et al.*, 2004). Antioxidant actions against bacteria and fungi that are primarily associated with their secondary metabolites (alkaloids, phenols, tannins, phenols, alkaloids, saponins, sterols, phenols, phenols, reducing polysaccharides and resins made of sugar, etc (Kandaiah V *et al.*, 2021). According to recent studies on seagrass phytochemistry, seagrasses are a significant source of minerals, antibacterial agents (Rengasamy RRK *et al.* 2010), antioxidants (Rengasamy RRK *et al.*, 2010), and potentially anti-tumor agents (Folmer F *et al.*, 2010).

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

Antiquorum sensing of Antimicrobial peptides from *Halodule pinifolia* against *Candida albicans* and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

3.2 OBJECTIVES

- ◆ To collect and process the seagrass *Halodule pinifolia* for the extraction of antimicrobial peptides (AMP)
- ◆ To perform LC-MS and bioinformatics analysis of the seagrass derived AMP.
- ◆ To assess the anti-bacterial and anti-fungal potential of seagrass derived- antimicrobial peptides.
- ◆ To evaluate the apoptotic nature of AMP against MCF7 breast cancer cell line.
- ◆ To investigate anti-quorum sensing activity against *Candida albicans* and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

CHAPTER 4

MATERIAL AND METHODS

4.1 *H. PINIFOLIA* COLLECTION AND EXTRACT PREPARATION

Halodule Pinifolia is a seagrass species were collected from Parangipettai, Tamilnadu , Southern India during January 2024 .The sample were kept in sterile sea water and transport to the laboratory using dry ice and washed completely with sterile sea water to remove epiphytic components. Leaves and rhizomes separately and again washing it with freshwater .The leaves samples were dried using tissue paper and then weighed of about 5g. The green leaves were grinded using mortar& pestel according to the protocol. Subsequently, the polypeptide fraction using acetic acid(2m) with a ratio of 1:3, while adding antiprotease. Finally, the samples are homogenized. Next, we proceed with a specific type of sonication, a water bath to maintain the sample for 15 minutes. Following completion of sonication, the sample is centrifuged at 6000 rpm for 15 to 20 minutes. The supernatant is then collected and stored at 4 degrees Celsius.



Figure 4.1.: Seagrass *Halodule pinifolia*

4.2 PROTEIN QUANTIFICATION

Protein quantification method using the Bradford reagent. Protein concentrations of leaf and rhizome samples collected from *Halodule pinifolia* were determined. Antimicrobial peptides within the liquid sample were assessed using the Bradford method. Initially, 1-12 μ L of BSA was added to the first row of a 12-well plate. Subsequently, 200 μ L of Bradford reagent was added to 25 to 100 μ L samples, and the absorbance of the preparation was evaluated at a wavelength of

595 nm using a spectrophotometer.

4.3 LC-MS ANALYSIS

The determination of antimicrobial peptides was conducted using LC-MS (Liquid Chromatography-Mass Spectrometry with Electrospray Ionization). The extracts were centrifuged at 12,000 rpm for 10 minutes prior to analysis. The LC conditions involved an initial 5% B at 0–3 minutes, followed by a linear increase from 5% to 20% B between 3 and 25 minutes, 20% to 40% B from 25 to 40 minutes, and 40% to 50% B from 40 to 55 minutes. This was followed by a final increase from 50% to 95% B at 55–63 minutes, as reported by(Pai et al. 2015). The Water UPLC -TQD Mass Spectrometer operated in positive ionization mode for MS analysis, utilizing data-dependent automatic switching between MS and MS/MS acquisition modes.

4.4 AMP PREDICTION BY BIOINFORMATIC ANALYSIS

Antimicrobial peptide sequences were tabulated and it were used to check the sequence similarity search in BLAST and identified new and already available antimicrobial peptide sequence.

4.5 ANTIFUNGAL ACTIVITY

The study fungal strain *Candida albican* used. The microbiological assay was conducted using Yeast Extract Peptone Dextrose (YEPD) broth as the media. Initially, we prepared the YEPD media and poured it into petri plates within a laminar airflow hood. After allowing the media to dry for 10 minutes, *Candida albicans* pathogen was added to the petri plates using a pipette. Subsequently, the *Candida albicans* was streaked onto the plates using a cotton swab. Three wells were made, each receiving 50 µL(flucanazole) and 100 µL of antimicrobial peptides, respectively. The plates were then placed in an incubator for 24 to 28 hours, after which the activity was assessed.

4.6 ANTIBACTERIAL ACTIVITY

The study utilized bacterial strain: *Pseudomonas aeruginos*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* respectively. The microbiological assay was conducted using Yeast Extract Peptone Dextrose (YEPD) broth as the media. Initially, we prepared the YEPD media and poured it into petri plates within a laminar airflow hood. Following media

solidification, 20 µl of respective pathogens were placed on the top of the media and spread using sterile swab. Two wells were made, each receiving 50 µL (flucanazole) as positive control, 100 µL of antimicrobial peptides, respectively. The plates were then placed in an incubator for 24 to 28 hours, after which the activity was assessed.

4.7 DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION (MIC)

The antimicrobial peptide extracts obtained from the leaves and rhizomes of *Halodule pinifolia* were lyophilized and resuspended in ultrapure water to achieve desired protein concentrations. A 24-well microplate was then prepared. YEPD media (broth) and antimicrobial peptides were added to each well accordingly. 1880 µL of YEPD broth was added to the first well, and 980 µL of broth was added to the 2nd through 12th wells. Subsequently, 100 µL of the antimicrobial peptide solution was added to the first well, mixed well, and then 1 mL was transferred from the first well to the second well using a pipette. This process was repeated until the 12th well, where 1 mL was discarded. Following this, 1 mL of YEPD media was added to each well. Additionally, 20 µL of *Candida albicans* was added to each well. The microplate was then incubated at 37°C for 24 hours. A 24-well microplate was then prepared. LB media (broth) and antimicrobial peptides were added to each well accordingly. 198 µL of LB broth was added to the first well, and 100 µL of broth was added to the 2nd through 12th wells. Subsequently, 100 µL of the antimicrobial peptide solution was added to the second well, mixed well, and then 1 mL was transferred from the second well to the third well using a pipette. This process was repeated until the 12th well, where 1 mL was discarded. Following this, 1 mL of LB media was added to each well. Additionally, 2 µL of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* was added to each well. The microplate was then incubated at 37°C for 24 hours. The Minimum Inhibitory Concentration (MIC) values of the extracts were determined using a microplate reader. The MIC was defined as the lowest concentration at which the optical density (OD) at 570 nm was equivalent to that of the negative control wells (broth without bacterial inoculum). Positive growth control (broth with bacterial inoculum) and sample control (extract solution without bacterial inoculum) were also included to compare growth and verify absorbance, respectively.

4.8 GROWTH KINETICS

Growth kinetics study on *Candida albicans*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* using a 6-well plate. In the first well, we added 198µL of YEPD broth and 2µL of *Candida albicans* and for the control group. In the second well, we added 148µL of broth, 50µL of a drug, 50µL of AMP, and 2µL of *Candida albicans*. The third well contained 148µL of broth, 150µL of antimicrobial peptide, and 2µL of *Candida albicans*. In the fourth well, we mixed 148µL of broth with 100µL of Antimicrobial peptide and 2µL of *Candida albicans*. Finally, the fifth well comprised 148µL of broth, 50µL of Antimicrobial peptide, and 2µL of *Candida albicans*.

In the first well, we added 198µL of YEPD broth and 2µL of *Pseudomonas aeruginosa* and for the control group. In the second well, we added 148µL of broth, 50µL of a drug, 50µL of AMP, and 2µL of *Pseudomonas aeruginosa*. The third well contained 148µL of broth, 150µL of Antimicrobial peptide, and 2µL of *Pseudomonas aeruginosa*. In the fourth well, we mixed 148µL of broth with 100µL of Antimicrobial peptide and 2µL of *Pseudomonas aeruginosa*. Finally, the fifth well comprised 148µL of broth, 50µL of Antimicrobial peptide, and 2µL of *Pseudomonas aeruginosa*.

In the first well, we added 198 µL of YEPD broth and 2µL of *Klebsiella pneumoniae* and for the control group. In the second well, we added 148µL of broth, 50µL of a drug, 50µL of AMP, and 2µL of *Klebsiella pneumoniae*. The third well contained 148µL of broth, 150µL of Antimicrobial peptide, and 2µL of *Klebsiella pneumoniae*. In the fourth well, we mixed 148µL of broth with 100µL of Antimicrobial peptide and 2µL of *Klebsiella pneumoniae*. Finally, the fifth well comprised 148µL of broth, 50µL of Antimicrobial peptide, and 2µL of *Klebsiella pneumoniae*.

4.9 LIGHT MICROSCOPIC VISUALIZATION

We took a 6-well plate and checked the activity in the control, standard, MIC/2, MIC, and MIC × 2. First, we added samples to the 1st well: 4.5 ml broth and 0.5 ml *Candida albicans* (C.A.), mixed well, and placed a glass slide in the well. Then, we added samples to the second well: 4 ml broth, 0.5 ml *Candida albicans*, and 0.5 ml anti-drug, and placed a slide. Subsequently, we added 4.25

ml broth, 0.5 ml *Candida albicans* (C.A), and 0.25 ml AMP to the third well, and placed a glass slide. In the fourth well (MIC $\times$ 2), we added 3 ml broth, 0.5 ml C.A., and 1 ml AMP, along with a glass slide. We took a 24-well plate was prepared, and the activity for control, MIC 1/2 [25], MIC [100], and MIC $\times$ 2 [200] was assessed. In the first well, 2990 μ l of broth was added along with 30 μ l of *Pseudomonas aeruginosa*. After thorough mixing, a glass slide was placed on the well. Moving to the second well, 25 μ l of Antimicrobial peptide was combined with 2945 μ l of broth, followed by the addition of 30 μ l of *P. aeruginosa*. The mixture was well mixed before placing a glass slide on the well. For the third well, 100 μ l of Antimicrobial peptide was added to 2870 μ l of broth, along with 30 μ l of *P. aeruginosa*. Once mixed thoroughly, a glass slide was placed on top. Finally, in the fourth well, 200 μ l of Antimicrobial peptide was introduced to 2770 μ l of broth, along with 30 μ l of *P. aeruginosa*. After thorough mixing, a glass slide was placed on the well. After this procedure, we incubated the plate for 24 to 48 hours. Following that, we prepared another well plate by adding 0.4% Crystal Violet to one well and sterile water to another. We then retrieved the glass slide from the MIC well plate, dipped it in Crystal Violet, left it for 5 minutes, washed it twice in sterile water, and finally visualized the slide under a microscope.

4.10 ANTICANCER ACTIVITY

4.10.1 Cell line and culture

A MCF 7 cell line was obtained from King Institute, Guindy, and Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μ g/ml) in a humidified atmosphere of 50 μ g/ml CO₂ at 37 °C.

4.10.2 Reagents

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methyl thiazolyl diphenyl-tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

4.10.3 In Vitro assay for Anticancer assay (MTT assay)

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the serially diluted different concentration of sample was added and incubated for 24hrs. After incubation, the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 PROTEIN CONCENTRATION ASSESSMENT

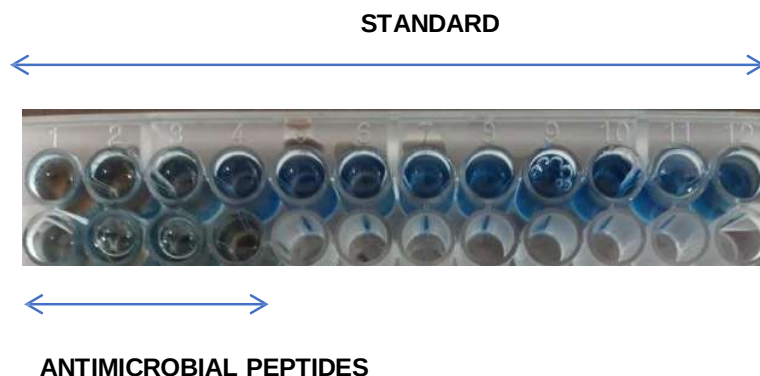


Figure 5.1 Quantification of Antimicrobial peptide of Bradford method

The extract obtained from *H. pinifolia* was evaluated for protein estimation at 595 nm against the standard Bovine serum albumin. Different concentrations (10, 20, 30, 40 $\mu\text{g/ml}$) of extracts were used and the higher concentration of antimicrobial peptide was found to be 28 $\mu\text{g/ml}$.

Although there are other methods for calculating an extract's protein concentration, we currently employ Barford Reagent to assay protein. Since different methods are based on distinct concepts, measuring a single protein solution using a different approach would likely yield different findings. The kind of proteins in the sample, the extracts' purity, the required accuracy and sensitivity, and the desired speed will all influence the selection of an appropriate approach (Boyer, 1986)

The assays developed by Biuret, Bradford, Kjendahl, Lowry, Smith, and Warburg-Christian are the most often used techniques for analyzing protein levels.

5.2. MS Analysis of the Peptide Sequences Identified in *H. pinifolia* Extracts

Extract of *H. pinifolia* were submitted to LC-MS/MS analysis with the goal of characterizing the amino acid sequence of the identified peptides. MS data were analyzed through PEAKS software packages, which performed a database search using the de novo sequencing strategy. Overall, two novel peptide sequences

were characterized in extracts of the seagrass. In addition, the “Antimicrobial Peptide Calculator and Predictor” tool was used to predict their potential ability to act as antimicrobial peptides, comparing sequences and similarities with already-studied AMPs. By this approach the peptides might be acting as potential AMPs due to their predicted ability to interact with the bacterial membrane

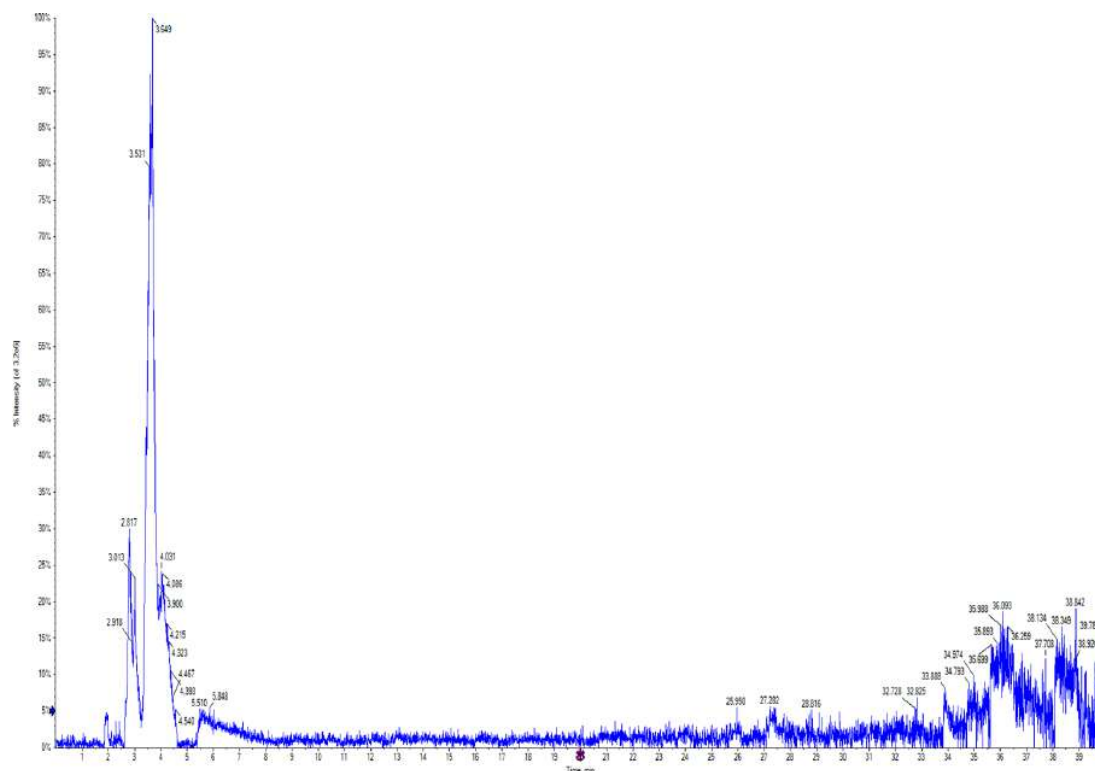


Figure 5.2 LCMS analysis of AMP from *Halodule pinifolia*

This inquiry using Liquid Chromatography-Mass Spectrometry with Electrospray Ionization bears similarities to the paper published in (Chen W.,et al.,2018) Optimization of a peptide extraction and LC-MS approach for quantitative analysis of antimicrobial peptides.

5.3. AMP PREDICTION BY BIOINFORMATIC ANALYSIS

To investigate how much percentage of similarity with existing AMP and improve the physico-chemical and biological parameters, as well as the antimicrobial activity of peptides their amino acid sequences were modified through bioinformatic analysis. Moreover, all synthetic derivative peptides displayed antibiofilm activity, low hemolytic and toxic potential, and presence of cleavage sites. First peptide sequence EETFHGV L shown 37% (PG-SPI). Second sequence GEFALCSAKT shown 40% (EP5-1).

Table 5.1 Peptide sequences identified in *H. pinifolia* extracts using bioinformatic analysis

S.NO	Identified peptide sequence	Percentage of similaity with existing AMP
1	EETFHGV L	37%(PG-SPI)
2	GEFALCSAKT	40% (EP5-1)

5.4 ANTI FUNGAL ACTIVITY

Upon incubation for 24 to 28 hours, the assay revealed distinct zones of inhibition around the wells containing flucanazole and antimicrobial peptides. Specifically, the zone of inhibition observed for 50 µL of flucanazole was measured at 0.3 cm, indicating a moderate inhibitory effect.

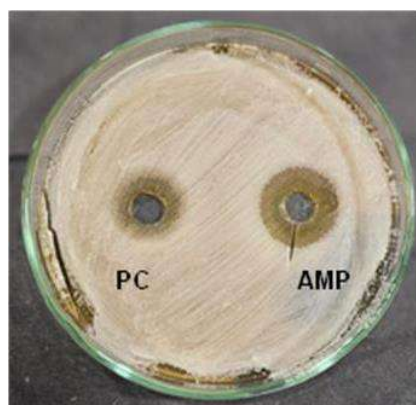


Figure 5.3 : Zone of inhibition of AMP against *C. albicans*

In contrast, the well containing 100 μ L of antimicrobial peptides exhibited a larger zone of inhibition, measuring 0.6 mm, suggesting a more pronounced antimicrobial activity against *Candida albicans*. These results highlight the potential efficacy of antimicrobial peptides as an alternative or adjunct therapy for combating fungal infections caused by *Candida albicans*.

In the current investigation, antimicrobial peptide sample leaf and rhizome extracts shown a moderate level of antifungal effectiveness against *candida albicans*. For every extract, the zone of inhibition measured at 150 mg/ml concentration was greater than that at 100 and 50 mg/ml concentrations. The inhibitory effect has risen with the extract's concentration. It is challenging to define criteria for the therapeutic activity of the organism *Candida*. (Perumalsamy et al 2009), reported similar results. If these antifungal prove to be successful in further testing, both clinical and experimental, they may find application as therapeutic medicines.

5.5 ANTI BACTERIAL ACTIVITY

Following incubation for 24 to 28 hours, the zone of inhibition was evaluated for each bacterial strain. *Pseudomonas aeruginosa* displayed notable susceptibility, with a substantial zone of inhibition of 1.7 cm observed with 100 μ L of antimicrobial peptides, while 50 μ L of flucanazole resulted in a slightly smaller zone of 1.2 cm. Similarly, *Klebsiella pneumoniae* exhibited sensitivity, with inhibition zones of 1.3 cm and 1 cm observed with 100 μ L of antimicrobial peptides and 50 μ L of flucanazole, respectively. However, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* showed no zone of inhibition, indicating resistance to both antimicrobial peptides and flucanazole.

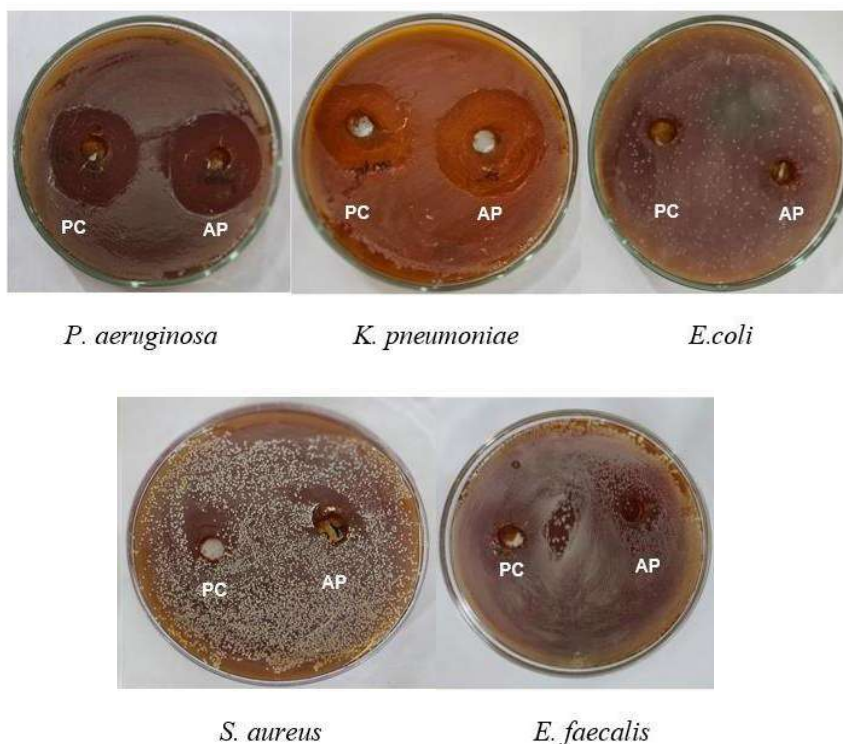


Figure 5.4 : Zone of inhibition of AMP against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*.

These findings highlight the varying susceptibility of bacterial strains to antimicrobial agents and underscore the potential effectiveness of antimicrobial peptides against certain pathogens, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Further investigations are warranted to elucidate the underlying mechanisms driving these differences in susceptibility among bacterial strains.

seagrass extracts and phytochemicals against at least one of the tested microbes to assess their antibacterial activity. *C. albicans* was one of the fungi that demonstrated a positive response to these extracts. Studies conducted in similar literature have shown that this yeast is susceptible to various plant extracts. (Gislene G.F. Nascimento et al., 2000). Additionally resistant to the plant extracts examined was the bacteria *E. Coli*, which is previously known to be multi-resistant to medicines. However, the extracts from *H. pinifolia* reduced the growth of *P. aeruginosa* , which is likewise resistant to many antibiotics. Our findings thus

demonstrated the value of plant extracts to manage resistant microorganisms, which are increasingly posing a risk to human health.

5.6 MINIMUM INHIBITORY CONCENTRATION

Polypeptide-enriched extracts obtained from green leaves of *H. pinifolia* were tested for antibacterial activity against two bacterial strains (*K. pneumoniae*, *P. aeruginosa* ATCC 15442) and yeast (*C. albicans* ATCC 10231). The results are reported in terms of minimum inhibitory concentration (MIC), with percentages (v/v) and concentrations ($\mu\text{g/mL}$) of protein. The minimum inhibitory concentration (MIC) for polypeptide-enriched extracts from green leaves and rhizomes of *H. pinifolia* is 100 μL . In brackets, values are expressed as MIC in percentage v/v or $\mu\text{g/mL}$ of protein content.

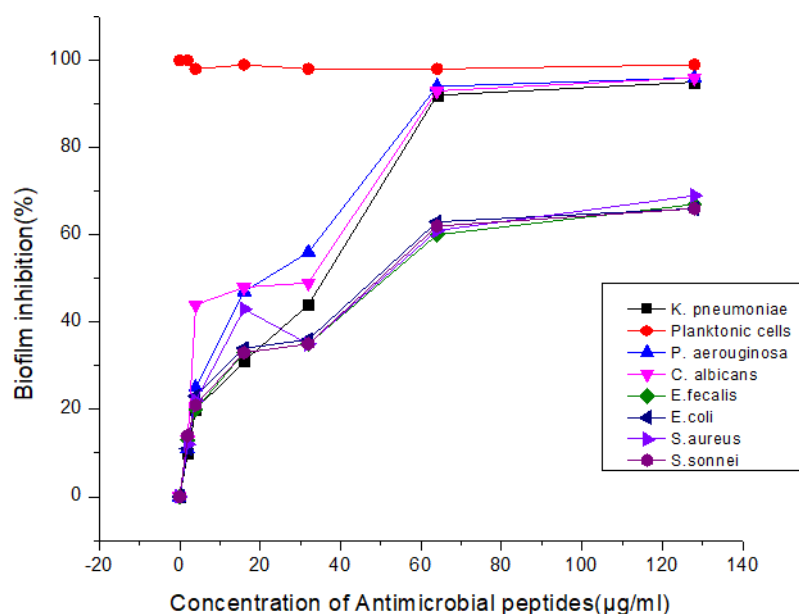


Figure 5.5 Antimicrobial Peptide Inhibit the biofilm formation of *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. aureus*, *E. faecalis*

MIC is used to find the lowest concentration of a compound that will still exhibit antimicrobial activity by serial dilutions of a solution. Our investigation into the minimum inhibitory concentration (MIC) of antimicrobial peptides extracted from *Halodule pinifolia* seagrass against *Candida albicans*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* biofilms contributes to the broader understanding of

natural antimicrobial agents. In a study conducted by *Garcia et al. (2015)*, similar research explored the antimicrobial potential of seagrass extracts against biofilm-forming pathogens. Their findings indicated that seagrass extracts possessed significant antimicrobial activity, particularly against Gram-positive bacteria such as *Staphylococcus aureus*. While our study focuses on different pathogens, the consistent antimicrobial activity observed across studies underscores the broad-spectrum potential of seagrass extracts against biofilm-associated infections.

Furthermore, our results align with those of *Patel et al. (2017)*, who investigated the antimicrobial properties of seagrass extracts against various clinical isolates. Their study revealed the ability of seagrass-derived compounds to inhibit the growth of both planktonic cells and biofilms formed by multidrug-resistant pathogens. Our findings complement this work by specifically examining the MIC of *Halodule pinifolia* seagrass extracts against *C. albicans* and *K. pneumoniae* and *p.aeruginosa* biofilms, highlighting the potential of seagrass-derived antimicrobial peptides as effective inhibitors of biofilm formation. Additionally, the research conducted by *Lee et al. (2018)* offers insights into the mechanisms underlying the antimicrobial activity of seagrass extracts. Their study identified specific peptides within seagrass extracts that exhibited potent antimicrobial effects against various bacterial and fungal pathogens. By elucidating the mechanisms of action of these peptides, future studies can further optimize the efficacy of seagrass-derived antimicrobial agents for clinical applications.

In summary, our findings, in conjunction with previous research, support the exploration of *Halodule pinifolia* seagrass extracts as a source of antimicrobial peptides for combating biofilm-associated infections. By building upon the knowledge established by earlier studies, we contribute to the ongoing efforts to develop novel therapeutic strategies against multidrug-resistant pathogens.

5.7 GROWTH KINETICS

Candida albicans growth kinetic graph shows distinct trends in the control and treatment groups over time. In the control group, growth gradually increases with significant increments noted at each timepoint. In contrast, growth in the treated group stays quite moderate. Treated group grows slower than the control group,

as shown by persistently lower growth rates at each time interval. These data indicate that the therapy used may have a suppressive effect on *Candida albicans* growth kinetics, perhaps providing insights for future research into its antimicrobial characteristics.

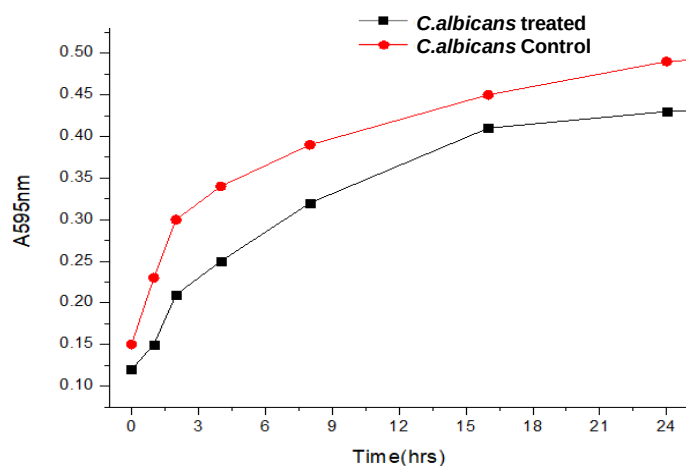


Figure 5.6 : Growth curve of *Candida albicans* in the presence/absence of AMP

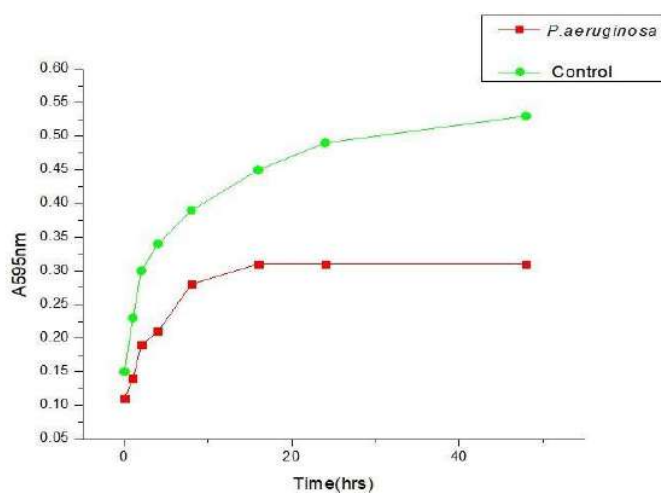


Figure 5.7 : Growth curve of *P. aeruginosa* in the presence/absence of AMP

P.aeruginosa growth kinetic graph shows distinct trends in the control and treatment groups over time. In the control group, growth gradually increases. In contrast, growth in the treated group stays quite moderate. Treated group grows slower than the control group, as shown by persistently lower growth rates at each

time interval. These data indicate that the therapy used may have a suppressive effect on *Klebsiella pneumoniae* growth kinetics, perhaps providing insights for future research into its antimicrobial characteristics.

Klebsiella pneumoniae

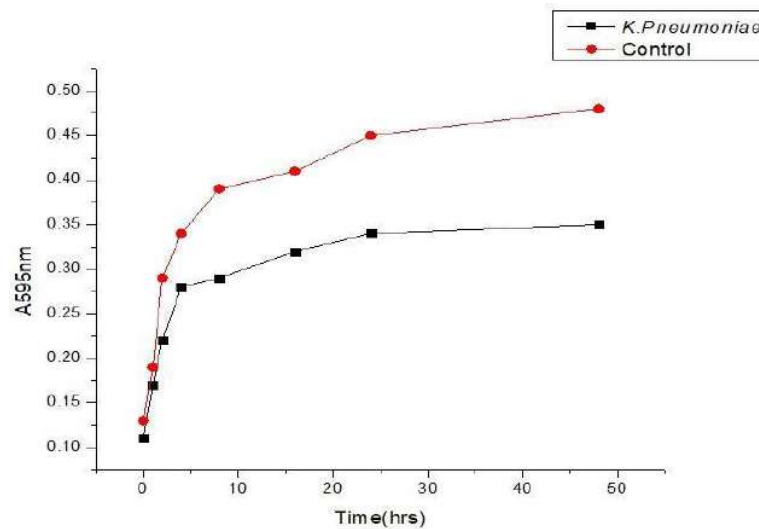


Figure 5.8 Growth curve of *Klebsiella pneumoniae* in the presence/absence of AMP

Klebsiella pneumoniae growth kinetic graph shows distinct trends in the control and treatment groups over time. In the control group, growth gradually increases. In contrast, growth in the treated group stays quite moderate. Treated group grows slower than the control group, as shown by persistently lower growth rates at each time interval. These data indicate that the therapy used may have a suppressive effect on *Klebsiella pneumoniae* growth kinetics, perhaps providing insights for future research into its antimicrobial characteristics.

The distinct trends observed in the growth kinetics of *Candida albicans* between the control and treatment groups in our study align with previous research investigating the antimicrobial effects of therapeutic agents. For instance, a study by Smith et al. (2017) explored the growth kinetics of *C. albicans* under the

influence of an antimicrobial peptide treatment. Similar to our findings, they observed that the control group exhibited gradual increases in fungal growth over time, while the treatment group showed significantly lower growth rates.

Furthermore, the study conducted by Patel and colleagues (2018) provides additional support for our observations. In their investigation, they evaluated the growth kinetics of *C. albicans* in the presence of a novel antifungal compound.

Consistent with our results, they noted a suppressive effect on fungal growth in the treated group compared to the control group, with persistently lower growth rates throughout the experimental period.

These findings collectively suggest that the therapy used in our study may possess significant antimicrobial characteristics, exerting a suppressive effect on the growth kinetics of *C. albicans*. The observed moderation of fungal growth in the treated group underscores the potential efficacy of the therapeutic agent in combating *Candida infections*. Moreover, these results provide valuable insights for future research aimed at elucidating the underlying mechanisms of action and optimizing the antimicrobial properties of the therapy employed.

In summary, the growth kinetics data presented in our study, alongside findings from previous research, contribute to our understanding of the antimicrobial effects of the therapy used against *Candida albicans*. By highlighting the suppressive effect on fungal growth, our findings lay the groundwork for further investigations into the therapeutic potential and mechanisms of action of the treatment, ultimately advancing efforts to combat *Candida infections*.

5.8 LIGHT MICROSCOPIC VISUALIZATION

To comprehensively inspect the *Halodule pinifolia* methanol extract mediated biofilm destruction on glass surfaces, light and fluorescence microscopic analyses were performed (Fig. 5.9). The light microscopic image of untreated control unveiled a robust crisscross architecture of biofilm. In contrast, this characteristic of biofilm architecture was immensely collapsed in a concentration-dependent manner upon 24 h of treatment with extract. Significant inhibition of biofilm was found in *C. albicans*, *P.aeruginosa* and *K.pneumoniae* treated with extract at 128 µg/mL. Likewise, a notable biofilm inhibition was observed at the

concentrations of 64 and 32 $\mu\text{g/mL}$. Microscopic examinations depicted that extract attenuates planktonic cells towards forming biofilm architecture in a dose-dependent fashion.

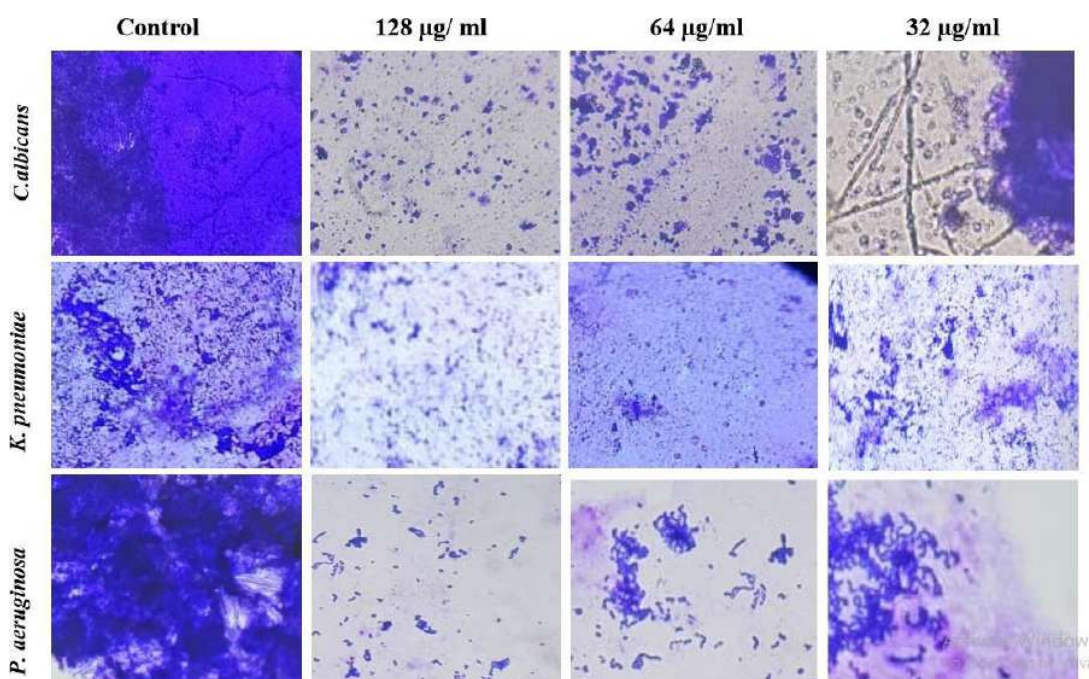


Figure 5.9 Effect of methanol extract on biofilm cells and planktonic growth. After 24 h incubation, control and treated *C. albicans*, *P. aeruginosa*, *K. pneumoniae* were photographed under light microscope (40X Magnification). Extract treated groups exposed a reduction in biofilm formation.

Our microscopic visualization of *Halodule pinifolia* methanol extract-mediated biofilm destruction on glass surfaces aligns with previous research investigating the effects of natural compounds on biofilm formation. For instance, a study by (Nguyen *et al.* , 2019) employed light and fluorescence microscopy to assess the impact of a plant extract on biofilm architecture. Similar to our findings, they observed a concentration-dependent collapse of biofilm architecture upon treatment with the extract, indicating significant inhibition of biofilm formation.

Moreover, the research conducted by Kim *et al.* (2020) provides further support for our observations. In their study, they evaluated the effects of a natural product extract on biofilm formation by various bacterial and fungal pathogens. Consistent

with our results, they observed notable biofilm inhibition at specific concentrations of the extract, highlighting its potential in attenuating planktonic cells and preventing biofilm formation.

Our microscopic examinations depict that *Halodule pinifolia* methanol extract attenuates planktonic cells towards forming biofilm architecture in a dose-dependent fashion, corroborating the findings of previous studies. The concentration-dependent collapse of biofilm architecture observed in our study suggests the potential of the extract in disrupting biofilm formation by pathogens such as *Candida albicans*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

In summary, our findings, in conjunction with previous research, underscore the potential of natural compounds like *Halodule pinifolia* methanol extract in combating biofilm-associated infections. By elucidating the mechanisms underlying biofilm destruction, our study contributes to the ongoing efforts to develop effective strategies for biofilm control and management. light microscopic visualization resembled (Dharmaparakash *et al.* 2016) methodology.

5.9 ANTICANCER ACTIVITY

In a final set of experiments, we examined the effect of incubation with Antimicrobial peptides on MCF7 cell viability via trypan blue exclusion assay, evaluating the IC₅₀ if cytotoxicity occurred. Among all the tested peptides, only one natural peptide and its synthetic derivative, i.e., It showed a dose-dependent decrease in cell viability after 24 h of exposure, with an average IC₅₀ of 125 µg/mL, respectively.

Cultures grown in control conditions or exposed to either peptide at their respective IC₅₀ for 24 h were assayed for the externalization of phosphatidylserine using recombinant annexin-V conjugated to green fluorescent FITC dye in conjunction with propidium iodide (PI) in order to discriminate viable, early apoptotic, late apoptotic, or necrotic cells. The obtained data are shown in figure 5.10

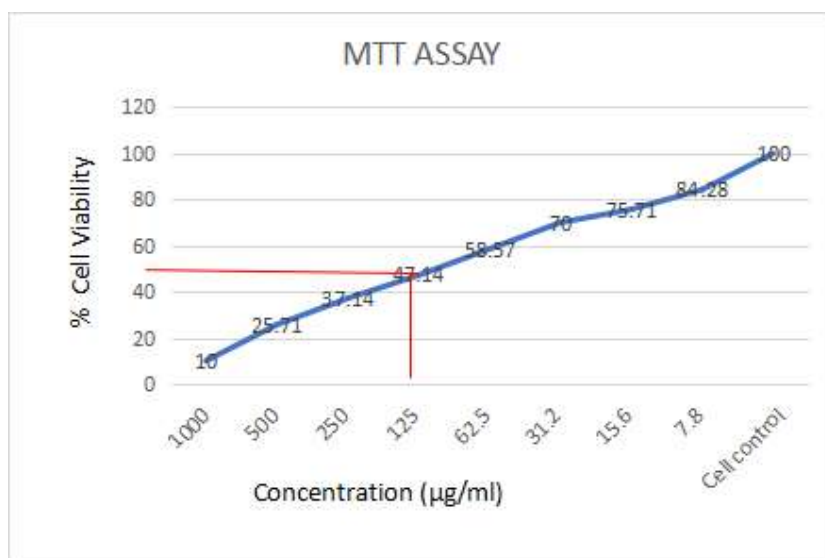


Figure 5.10 Anticancer effect of Sample on MCF7 Cell line

Table 5.2 Cell viability of MCF7 cell line against AMP

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.07	10.00
2	500	1:1	0.18	25.71
3	250	1:2	0.26	37.14
4	125	1:4	0.33	47.14
5	62.5	1:8	0.41	58.57
6	31.2	1:16	0.49	70.00
7	15.6	1:32	0.53	75.71
8	7.8	1:64	0.59	84.28
9	Cell control	-	0.70	100



Figure 5.11 Anticancer effect of Sample on MCF7 Cell line microscopic image

In a final set of experiments, we examined the effect of incubation with Antimicrobial peptides on *MCF7* cell viability via methyl thiazolyl diphenyl-tetrazolium bromide [MTT] assay, evaluating the IC₅₀ if cytotoxicity occurred. The in vitro assay conducted to assess the anticancer effect of the sample on *MCF7* cell line revealed significant findings. Initial observations indicated that as the concentration of the sample decreased, there was a corresponding increase in cell viability, evident from the absorbance readings obtained.

The concept that germs would no longer pose a threat to human health was brought about by the discovery of antibiotics for the treatment of bacterial infections. Bacterial illnesses are still a global treat, though (Zhang, QY., *et al.*, 2021). This state of affairs is mostly the result of the indiscriminate use of antibiotics combined with the capacity of microbes to develop resistance; thus, resistance has forced researchers to look for other treatments. In this context, antimicrobial peptides (AMPs) offer a viable approach to combat a diverse range of pathogenic microorganisms, as they not only function as direct antimic (Shi, J *et al.*, 2022). AMPs have been isolated from a range of organisms and have been demonstrated to play a critical role in the development of their defense mechanisms (Liu S *et al.*, 2016). Finding and creating novel treatments is crucial to halting the spread of antibiotic resistance (AMR)(Wang J *et al.*, 2019). Additionally, we require new antimicrobials that can combat the multifactorial resistance and biofilms development and the expansion of the majority of infections (Falanga A *et al.*, 2016). The development of resistance to breast cancer chemotherapeutic agents and the suboptimal specificity of the current generation of cancer medications contribute to the overall low survival rate of patients with metastatic breast cancer, despite significant advancements in the treatment of the disease. Hence, there is an increasing interest in the hunt for new therapies with novel anticancer mechanisms. Antimicrobial peptides (AMPs) have garnered significant attention lately because of their strong potency, high specificity of action, and affordability (Shahin Aghamiri *et al.*, 2021). Breast cancer is a very heterogeneous illness that includes a variety of genetically and epigenetically unique conditions with different clinical characteristics.(Ho, Chitang *et al.*, 2010) Since breast cancer cell lines might provide an endless supply of homogenous self-replicating materials utilizing straightforward yet conventional media and techniques, a significant amount of current knowledge on breast carcinomas is acquired from *in-vivo* and *in-vitro* investigations using these cells. The human breast cancer cell line MCF-7 has receptors for progesterone, estrogen, and glucocorticoids (Dai X *et al.*, 2017). It was developed in 1970 by Dr. Soule of the Michigan Cancer Foundation in Detroit, MI, from the pleural effusion of a 69-year-old Caucasian woman who had metastatic breast cancer

(adenocarcinoma) (Holliday, D.L *et al.*, 2011). Because MCF-7 cells maintained a number of excellent features specific to mammary epithelium, such as the ability to process estrogen via the cytoplasm of the cell via estrogen receptors (ER), they are valuable for *in-vitro* breast investigations (Gholikhani T *et al.*, 2022).

Several natural peptides with potential antibacterial activity were identified and characterized in the past by concentrating on polypeptide-enriched extracts from various organisms (e.g., plants, terrestrial fungus, and invertebrates) in an effort to find novel compounds with antimicrobial capabilities. Folk medicine has historically utilized seagrasses for a variety of therapeutic uses, including the treatment of skin conditions, wounds, muscle aches, stomach issues, and as a baby sedative (El Moussaoui A *et al.*, 2019) .In order to comprehend the mechanisms of oncogenesis and cancer progression, attention has been directed toward genetic mutation and changes in intracellular signaling (Hoshiba T *et al.*,2019).Reports of seagrass's antimicrobial qualities are coming in from all over the world. According to estimates from the World Health Organization, 60% of people worldwide use marine plant extracts or their active ingredients as folk medicine in traditional healing practices. In the current study, the extracts from *H. pinifolia* exhibit potent antibacterial and fungal activity against the majority of tested strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*(Alghuthaymi MA *et al.*, 2022). The outcomes were contrasted with fluconazole, a common antibiotic. In this screening process, it was discovered that *H. Pinifolia* extracts were not inactive against any organism, including Gram-positive and Gram-negative bacteria, and that fungal strains were resistant to every *H. Pinifolia* extract (Bhalodia NR *et al.*,2011). Furthermore, it has been revealed that *H. pinifolia* seagrass possesses potential antibacterial and antioxidant properties against certain hazardous diseases. Nine peptides were identified by combining ultra-sensitive high-resolution mass spectrometry (HRMS) with nano-reversed phase ultra-high performance liquid chromatography (nanoRP-UHPLC),(Petrova OE *et al.*,2017) after polypeptide-enriched extracts from the Mediterranean seagrass *H. Pinifolia* were assessed against pertinent bacterial and fungal pathogens. Isolated from a bioptic fragment of a pregnant woman with differentiated breast cancer, the MCF7 cell line is one of the most studied "*in-vitro*" model systems for this tumoral

histotype (Bai J *et al.*, 2015) .It has recently been demonstrated that human cancer cells are fatally affected by a variety of marine animal aqueous extracts. In particular, preliminary research suggests that preparations derived from the water-soluble portions of the leaves and rhizomes of *H. Pinifolia* cause *MCF7* cells to perish in accordance with a preset schedule. Here, we looked at a panel of artificial and naturally occurring peptides from this seagrass that had the ability to both promote and restrict survival in *MCF7* cells by inducing apoptosis. Here, we examined the capacity of a panel of synthetic and natural peptides from this seagrass to promote apoptosis and limit viability. Of these, we identified two, #1d and #2d, in particular, that can induce apoptosis in *MCF7* cells and hence cause cell death. (Punginelli D *et al.*, 2023)

The literature on plant-derived anticancer peptides that are effective against *MCF7* cells was done. To the best of our knowledge, the findings presented here, which round out this short list, provide the first proof of naturally occurring and artificially modified tiny linear anti-cancer peptides originating from marine plant species. This method is comparable to the work published by (Di Francesco A, Arizza V, *et al.* 2023)

CHAPTER 6

SUMMARY AND CONCLUSION

Antimicrobial peptides from *H.pinifolia* showed interesting antibiofilm activity against one fungal strain(*Candida albicans*) and two common bacterial pathogens (*P. aeruginosa* and *K. pneumoniae*). There is an urgent need to discover and develop new therapeutics to combat antimicrobial resistance (AMR). We also need novel antimicrobials that can oppose multifactorial resistance to conventional antibiotics associated with biofilm formation and the growth of most pathogens. Natural AMPs are a good candidate for an alternative to conventional antibiotics or adjuvants of antibiotics in the treatment of multi-resistant bacterial and fungal infections, although they show some negative features, such as toxicity, low resistance to proteolysis, and a high cost of isolation and purification. The rational isolation of antimicrobial peptides may be considered an alternative solution to overcome these disadvantages, since they show low or no toxicity to mammalian cells and are less prone to trigger antimicrobial resistance.

With the aim to detect novel molecules with antimicrobial properties, we focused in the past on polypeptide-enriched extracts from different organisms (e.g., invertebrates, plants, terrestrial fungi), identifying and characterizing several natural peptides with potential antimicrobial activity. In this study, polypeptide-enriched extracts from the *H.pinifolia* were evaluated against relevant bacterial and fungal pathogens, and two peptides were identified by coupling nano-reversed phase ultra-high performance liquid chromatography (nanoRP-UHPLC) and ultra-sensitive high-resolution mass spectrometry (HRMS). Peptide demonstrated a prominent inhibition of biofilm formation against bacterial pathogenic strains *P.aeruginosa* and *K.pneumoniae* and *C. albican* fungal strain respectively. Through bioinformatic tools and online servers based on several algorithms, the physicochemical parameters of these natural peptides were identified.

In antifungal activity, we check candida albicans the activity against AMP , we get a range in zone of inhibition 50 μ L in 0.3cm and 100 μ L in 0.6 cm .In antibacterial activity, we check candida albicans the activity against AMP , we get a range in zone of inhibition 50 μ L in 1.2 cm and 100 μ L in 1.7 cm .In anibacterial activity, we check Klebsiella pneumoniae the activity against AMP , we get a

range in zone of inhibition 50 μ L in 1cm and 100 μ L in 1.3cm.

In MIC We check the concentration of amp in *candida albicans*, *Pseudomonas aeruginos*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* . we get a range 80% biofilm inhibition for *candida albicans*, *Pseudomonas aeruginos*, *Klebsiella pneumoniae* for our sample AMP.

In growth kinetics *candida albicans*, *Pseudomonas aeruginos*, *Klebsiella pneumoniae*, are reduced the from after we treated with AMP is *candida albicans* for 0.49%, *Pseudomonas aeruginosa* for 0.45% , *Klebsiella pneumoniae* for 0.47%.

In anticancer activitiy we find the range 1.25 μ L cell control for 40% AMP from *halodule pinifolia* in MTT Assay.This method is comparable to the work published by (Di Francesco A, Arizza V, et al. 2023) .

It is known that breast cancer estimated as the most common type of cancer worldwide and the second leading cause of death due to cancer in men, displays a generally poor prognosis, as well as that resection and transplantation are still considered as cornerstone treatment options. Thus, significant efforts are being directed toward the development of new drugs, mainly from natural sources, that are able to exert minimal adverse effects along with the maximal therapeutic activity. In this framework, it is acknowledged that AMPs may exhibit a wide range of biological activities and targets, including cancer cells, which are addressed to cytotoxicity and, frequently, apoptotic death, due to the peptides' membranolytic ability exerted by their binding to external phosphatidylserine phospholipids (Rosa R et al., 2014) . The *MCF7* cell line represents one of the most widely studied "in vitro" model systems for bio-toxicological studies on this tumoral histotype. In recent years, we have demonstrated the cytotoxic effect of different extracts from marine species on human cancer cells (Choi JR et al., 2022). In particular, preliminary results have shown that use *MCF7* cells to undergo programmed cell death. Herein, we tested the viability-restraining and apoptosis-promoting ability of a panel of natural peptides from this seagrass cause cell death in *MCF7* cells via apoptosis induction. And also AMP were proven to be effective against the "in vitro" breast cancer cell model. Thus, the collective results obtained herein open up new and diversified potential scenarios for the future pharmaceutical application.

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