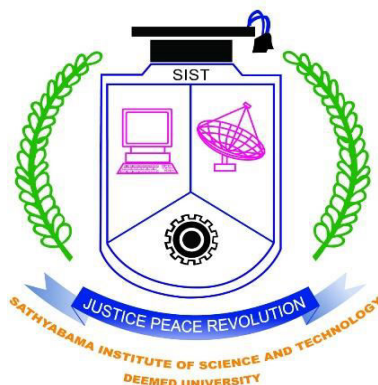


ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By
RAMYA. M (37230067)
KALARANJANI. K (37230033)



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

SATHYABAMA

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

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

APRIL-2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Ramya.M (37230067)** and **Kalaranjani.K (37230033)** who carried out the project entitled "**ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION**" under our supervision from **December 2020 to March 2021**.

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This is also to certify that the thesis represents the independent work of the candidate.

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(Research Guide)

Place : Parangipettai

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This is to certify that the thesis entitled “**ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION**” is a bonafide record of project work done by **Kalaranjani. K (37230033), B.Tech in Biotechnology, Sathyabama Institute of Science and Technology, Chennai** - 600119, under my guidance at our centre during the period from December 2020 – March 2021. This thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title to the candidate.

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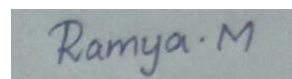
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DECLARATION

We **Ramya.M (37230067)** and **Kalaranjani.K (37230033)** hereby declare that the Project Report entitled “**ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION**” done by us under the guidance of Dr. M. Masilamani Selvam, Associate Professor (Internal) and Dr. M. Arumugam, Associate Professor (External) at Center of Advanced Study in Marine Biology, Annamalai University is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

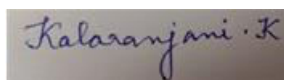
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PLACE: Chennai

2)

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SIGNATURE OF THE CANDIDATE

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ABSTRACT

Marine fouling organisms accumulate and deteriorate the surface of submerged ship. To overcome foul formation on the surface, there are so many antifouling paints available in the market which contains high toxic chemicals. When these toxic paints dissolve in water it may cause a harmful effect on marine organisms and demolish the ecosystem. Marine plant exhibits antifungal, antibacterial, antioxidant and antifouling activities. This research work mainly concentrates on replacing the toxic chemicals in the paint by incorporating eco-friendly natural component available in salt marsh. Hence, an attempt was done to evaluate the antifouling potential of salt marsh plants. In this study, methanolic extracts of three salt marsh plants, viz. *Ipomoea pes-caprae*, *Sesuvium portulacastrum* and *Batis maritima* were screened for antifouling activity against six marine fouling bacteria (*Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas sp.*, *Vibrio sp.*, *Staphylococcus aureus*, *Escherichia coli*) using disc diffusion method and these extracts were incorporated into commercial paint for field studies. Methanolic extract of *Ipomoea pes-caprae* (leaves) exhibited maximum zone of inhibition against *Pseudomonas sp.* The result of field study shows that sample-I (*Ipomoea pes-caprae*) mixed with paint coated on wood substrate has less foul formation compared to the control and other two samples. The result revealed that the salt marsh plant (leave) has potent antifouling activity, and it could be used as natural antifoulant in near future.

Keywords: Fouling organisms, toxic chemical, antifoulant, salt marsh plant.

TABLE OF CONTENTS

CHAPTER.NO	TITLE	PAGE NO.
	ABSTRACT	I
	ABBREVIATION	IV
	LIST OF FIGURES	V
	LIST OF TABLES	VII
1	INTRODUCTION	1
	1.1 Biofilm formation	1
	1.2 Different approaches to prevent fouling	2
	1.3 Salt marsh	3
	1.4 Outline of the project	4
2	LITERATURE SURVEY	5
3	AIM AND SCOPE	10
4	MATERIALS AND METHODS	11
	4.1 Materials	11
	4.2 Sample collection	12
	4.3 Solvent extraction	12
	4.4 Field trials	12
	4.4.1 Preparation of emulsion	12
	4.4.2 Preparation of raw materials	12
	4.4.3 Study area	12
	4.5 Antifouling assays	13
	4.5.1 Microorganisms and inoculum development	13
	4.5.2 Antifouling activity	13
	4.5.3 Minimum inhibitory concentration (MIC)	13

	4.6 FTIR spectral analysis	14
5	RESULTS AND DISCUSSION	15
	5.1 Sample collection	15
	5.2 Sample preparation	16
	5.3 Emulsion preparation	17
	5.4 Field study results	23
	5.4.1 After 15 days of observation	23
	5.4.2 After 31 days of observation	27
	5.5 Antifouling activity against marine fouling bacteria	29
	5.6 Minimum inhibitory concentration against marine fouling bacteria	36
	5.7 FTIR analysis	42
6	SUMMARY AND CONCLUSION	50
7	REFERENCES	51

LIST OF ABBREVIATIONS

FTIR	Fourier Transform Infrared Spectroscopy
UV	Ultra violet
g/l	gram per litre
RPM	Rotation per minute
mL	Milliliter
ppt	Parts per thousand
μL	Micro litre
MIC	Minimum inhibitory concentration
mg	Milligram
mm	Milliliter
cm	Centimeter
C	Carbon
Br	Bromide
N	Nitrogen
O	Oxygen
H	Hydrogen

LIST OF FIGURES

FIGURE	Title	Page No
5.1	<i>Ipomoea pes-caprae</i>	15
5.2	<i>Sesuvium portulacastrum</i>	15
5.3	<i>Batis maritima</i>	16
5.4	Samples were crushed by using motor and pestle method	16
5.5	Crushed samples kept in Shaker for incubation	16
5.6	Samples after incubation	17
5.7	Asian enamel paint and thinner	17
5.8	Paint and Thinner	18
5.9	Paint and Solvent	18
5.10	Paint and Sample I	18
5.11	Paint and Sample II	18
5.12	Paint and Sample III	18
5.13	Raw materials	19
5.14	Controls (uncoated substrates)	19
5.15	Paint and Thinner coated substrates	20
5.16	Paint and Solvent coated substrates	20
5.17	Paint and sample I coated substrates	20
5.18	Paint and sample II coated substrates	21
5.19	Paint and sample III coated substrates	21
5.20	The full picture of substrates before immersing at estuary	22
5.21	The tied substrates immersed at lowest low tide of the estuary	22
5.22	Shows site where the field study was performed	23
5.23	Uncoated substrates kept for control	23
5.24	Sample I and paint coated substrates (15 days)	24
5.25	Sample II and paint coated substrates (15 days)	24
5.26	Sample III and paint coated substrates (15 days)	25
5.27	Thinner and paint coated substrates (15 days)	25

5.28	Solvent and paint coated substrates (15 days)	26
5.29	Graph represents the foul formation on different substrates (controls) in percentage after 15 days of observation	26
5.30	Graph represents the foul formation on different substrates (sample coated) in percentage after 15 days of observation	27
5.31	The uncoated control	27
5.32	Sample I and paint coated substrates (31 days)	27
5.33	Sample II and paint coated substrates (31 days)	27
5.34	Sample III and paint coated substrates (31 days)	28
5.35	Solvent and paint coated substrates (31 days)	29
5.36	Thinner and paint coated substrates (31 days)	30
5.37	Graph represents the foul formation on different substrates (controls) in percentage after 31 days of observation	31
5.38	Graph represents the foul formation on different substrates (sample coated) in percentage after 31 days of observation	31
5.39	Plate without culture swab (control)	32
5.40	Zone of inhibition formed at various concentrations against <i>Porteus vulgaris</i>	33
5.41	Zone of inhibition formed at various concentrations against <i>Staphylococcus aureus</i>	34
5.42	Zone of inhibition formed at various concentrations against <i>Escherichia coli</i>	34
5.43	Zone of inhibition formed at various concentrations against <i>Porteus mirabilis</i>	35
5.44	Zone of inhibition formed at various concentrations against <i>Vibrio sp.</i>	35
5.45	Zone of inhibition formed at various concentration against <i>Pseudomonas sp.</i>	36
5.46	Turbidity level at different concentration against <i>Proteus Vulgaris</i>	38
5.47	Turbidity level at different concentration against <i>Staphylococcus aureus</i>	38

5.48	Turbidity level at different concentration against <i>Escherichia coli</i>	49
5.49	Turbidity level at different concentration against <i>Porteus mirabilis</i>	40
5.50	Turbidity level at different concentration against <i>Vibrio</i>	41
5.51	Turbidity level at different concentration against <i>Pseudomonas sp.</i>	41
5.52	Minimum inhibitory concentrations of <i>Escherichia coli</i>	42
5.53	Minimum inhibitory concentrations of <i>Proteus mirabilis</i>	42
5.54	Minimum inhibitory concentration of <i>Proteus vulgaris</i>	43
5.55	Minimum inhibitory concentration of <i>Pseudomonas</i>	43
5.56	Minimum inhibitory concentration of <i>Staphylococcus aureus</i>	44
5.57	Minimum inhibitory concentration of <i>Vibrio sp.</i>	44
5.58	FTIR analysis of methanolic extract <i>Ipomoea pes-caprae</i>	46
5.59	FTIR analysis of methanolic extract <i>Sesuvium portulacastrum</i>	46
5.60	FTIR analysis of methanolic extract <i>Batis maritima</i>	47

CHAPTER 1

1. INTRODUCTION

Marine biofouling is caused by the adhesion of micro and macro foulers on a submerged wet surface. The environmental factors that induce the growth of fouling organisms are temperature, turbidity, waves, salinity, light, current, tides, color, pollution and texture of the substrate. The marine micro foulers are bacteria, protozoans, spores of macroalgae, diatoms and macro foulers are algae, barnacles, bryozoans, cnidarians, annelids and ascidians. A bacterium plays a major role in biofilm formation that leads to the settlement of invertebrates, sessile plants and animals on underwater structures (Nandhini. S and K. Revathi, 2016). Fouling on ship tends to hydrodynamic drag and hull roughness. Due to this reduction in speed and maneuverability of ship occurs (Kirschner et al., 2012).

1.1 Biofilm formation:

Marine biofilm formation generally occurs in substrates for the colonization and settlement of larvae and spores of higher sessile organisms. These microbial communities initiate the attachment of the foulers on the surface by triggering the fouling process; the interaction and specific mechanism convoluted in the initial surface adhesion are still unknown. Biofilm itself have an important role in the corrosion of surfaces immersed in seawater (Antunes, et al., 2019). Marine biofouling remains a problem with significant economic and environmental deprivation of marine transportation are increased fuel consumption up to 30% to balance the standard level of performance and this increased consumption of fuel induces the release of greenhouse gases into the environment (Garcia, et al., 2020). Biofouling happens all over the world in various fishing equipment, offshore oil, gas industries and industries. Unguarded submerged marine metallic surfaces are mainly subjected to biofouling and corrosion (Doiron et al., 2018). The gradual fuel consumption leads to the provocation of CO₂ emission and more water transportation cost. (Abioye et al., 2019). The environmental consequences of transportation have given rise to many

initiatives like greener maritime shipping and reducing the emissions from ships which affects the air quality (Davidson et al., 2009).

1.2 Different approaches to prevent fouling:

Marine fouling can be removed by three different methods namely chemical, mechanical and biological methods. Chemical methods are the most commonly used technique which includes toxic antifouling paints, foul release coating and chlorination. The toxic antifouling paints are generally used to cover the surface of the boat or ship hulls with a layer of biocides. Antifouling paints are classified into soluble and insoluble matrix paints and self-polishing copolymer paints. The Soluble matrix paints contains a soluble polymer binder with a biocide, the polymers get dispersed into water as a result the biocides are release into sea water. In insoluble matrix paints the binder used is a hard insoluble polymer that does not erode on immersion into sea. But there is chance of dispersion of biocide into water only by the dissolution of biocide particles. Self-polishing copolymer paint consists of mainly an acrylic backbone polymer as a binder with an ester linked to organometallic group. These polymers are also dissolved into water by chemical reaction (Kiil, 2001). Foul-release coating contains anon-sticky property which reduces the adhesion of fouling organisms on the surface, which leads to the removal of fouls by hydrodynamic stress while navigation or just cleaning it mechanically. TBT is the most commonly used biocide. Antifouling paints incorporated with TBT (tributyl tin) was the first chemical found effective and durable against fouling organisms at 1960s. Though it is successful, due to its toxicity it harms non targeted marine organisms therefore it was banned by the government at 1970s (Lejars et al., 2012). The mechanical methods are surface modification, electro-chlorination and ultrasonic system. Surface modification is method similar to the natural organism's defence against bio adhesion. Structural changes and micro topographical features can be modified on the surfaces to prevent biofouling (Bers and Wahl, 2004). Electro-chlorination works on the principle of electrolysis which converts sodium chloride in the seawater into sodium hypochlorite solution to prevent biofouling (Sampathkumaran et al., 2008). Ultrasonic is device emitting mechanical waves in the ultrasonic (>20 kHz) and audible (20 Hz–20 kHz) frequency range leads to the prevention of biofouling (M. Legg et al., 2015). Some physical methods employed in biofouling are scraping method, tar and pitch (Wang et al., 2020). Biological method is the addition of non-

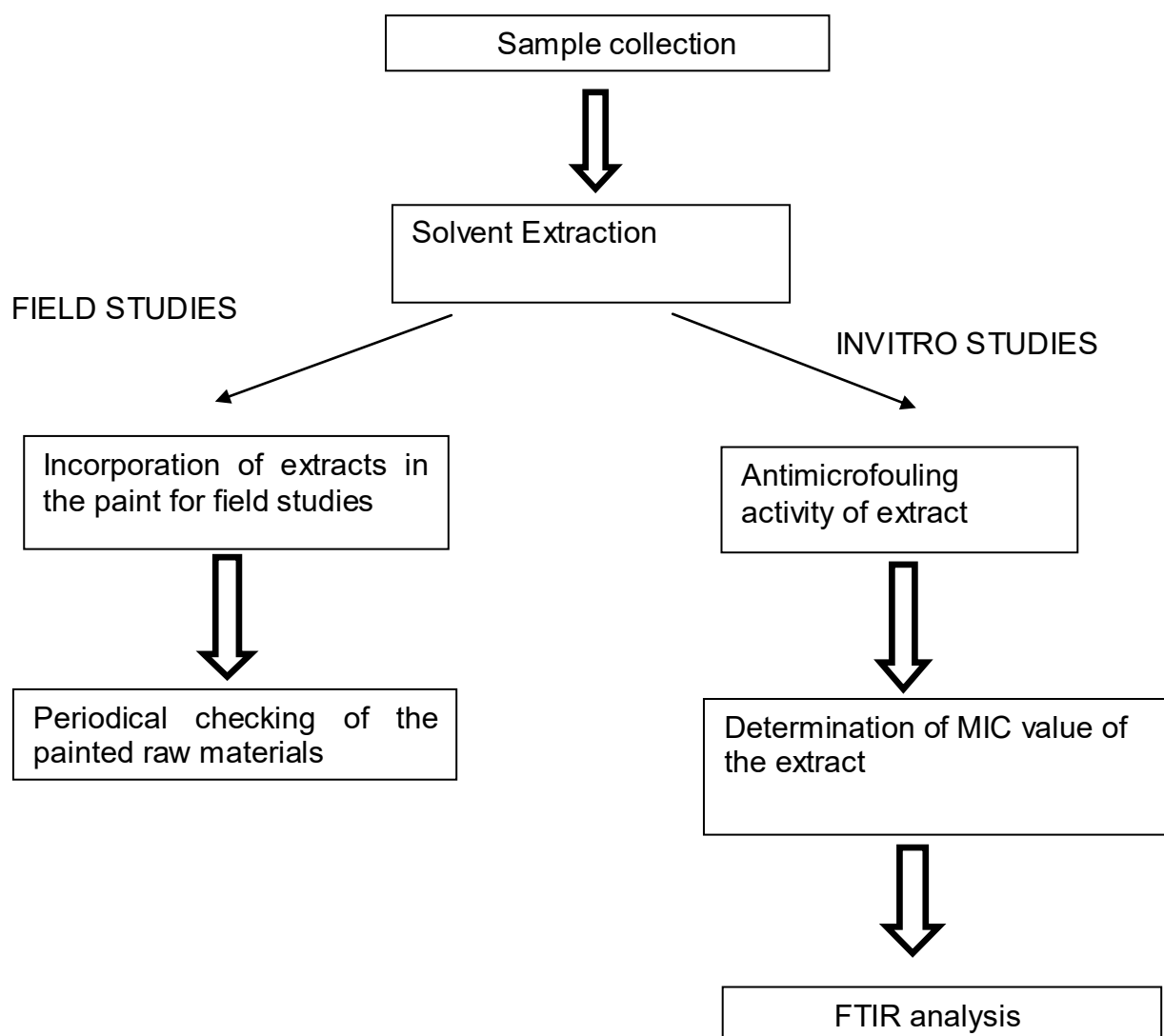
toxic or natural products from marine organisms into paint. Many marine animals are free from fouling and produce metabolites or antifouling agent which is used to prevent marine biofouling (Burgess et al., 2003).

In current scenario antifouling paints are available in the combinations of copper, zinc, tin and other heavy metals. To replace this toxic metal biogenic compounds are incorporated into the paints to produce an eco-friendly antifouling paint.

1.3 Salt marsh:

Plant cover was a primary aspect of many terrestrial systems, coastal marine and it also controls the structure of associated animal communities (Whitcraft et al., 2007). Marine plant exhibits antifungal, antibacterial, antioxidant and antifouling activities (khan et al., 2014). In those plants salt marshes diminishes the water waves and thus have an essential function for coastal protection. Biophysical properties of these plants play a major role in the activity of wave attenuation and may be distinguished by morphological properties such as vegetation and stem density (Schulze et al., 2019). Salt marshes are a highly valuable ecosystem which gives important to ecosystem. The global scale of marsh disappearance due to coastal squeeze and climate change, the factors affecting the edges of salt marsh by erosion are extrinsic factor such that foreshore morphology, wind exposure and the intrinsic factors are soil and vegetation (Wang et al., 2017). The occurrence of salt marsh landforms is of highly important as their connected ecosystems gives important to regulating, cultural ecosystem services and provisioning (Brooks et al., 2020). Salt marshes are widely distributed throughout the coastal area in the form of dense bunches, climbers and shrubs. The salt marsh takes up only H_2O from salt water for the growth and leave out the $NaCl$. It also highly tolerant to salt. The ability of salt marsh towards marine foulers needs to be tested. This study was done to assess antifouling activity from salt marsh towards marine foulers.

1.4 Outline of the project:



CHAPTER 2

2. LITERATURE SURVEY:

(Nandhini. S and K. Revathi, 2016) this work was done to evaluate the antifouling potential of two mangrove species against the collected biofoulant sample. This study has shown the result that mangroves have potent AF component which can be used as a natural antifoulant.

(Kirschner et al., 2012) This review summarizes the certain effects of biofouling and explains the basis for antifouling coating design and highlights prominent advances in antifouling technologies.

(Antunes et al., 2019) this paper describes the biofilm formation and the different composition of biofilm which is isolated from the different artificial submerged surfaces via bacterial quorum sensing.

(Davidson et al., 2009) this paper compares the previous studies of how much fouling organism forms on container ship than the other submerged vessels and the risk factor to the environment by fouling organism.

(Doiron, K et al., 2018) this study was to demonstrate the function of antimicrobial peptides extracted from snow crab (SCAMPs) to reduce the formation of marine biofilms on immersed mild steel surfaces. Hence five antimicrobial peptides were found in the snow crab hydrolysate fractions were used. SCAMPs were demonstrated to interact with natural organic matter during the formation of the conditioning film and to limit the marine biofilm development in terms of viability and bacterial structure.

(García et al., 2020) this paper indicates the effective tool to study biofouling on hull properties by using four different fouling resistant coatings which were experimentally investigated to increase the hydrodynamic performance of the ship this can cause decrease in fuel consumption.

(Khan et al., 2001) this paper mainly concentrates on the health issue of

ornamental fish for its sustainable growth. Therefore, five different marsh plants were taken which has high medicinal values and tested against six fish pathogens isolated from aquarium.

(Schulze et al., 2019) this research work describes the biomass cultivation and efficiency of salt marsh that can attenuate the wave for the protection of coastal region.

(Wang et al., 2017) this paper talks about the factors that causing salt marsh erosion and the quantification of erodibility of sediment cores collected from the marsh edge of various salt marsh plants was done experimentally.

(Whitcraft et al., 2007) this paper says that the factors which affecting the salt marsh plant consequently such that habitat degradation, sea level, climate change and marsh restoration.

(Chambers et al., 2014) this author has done field trials for natural product coated paint. Electrochemical technique method was used to investigate the effectiveness of the paint.

(Soren Kiil, 2001) this paper is about the development of self-polishing antifouling paints in a mathematical model. The model paints were prepared in different composition using copolymers and pigments. The experiment was conducted using a laboratory rotor to check the polishing rate and the amount of pigment leached by the interaction between the seawater and the artificial paint prepared.

(Lejars et al., 2012) this review paper is on foul-release coating a non-toxic antifouling coating. Due to the use of biocide or toxic AF coating is used to find an alternative to this, a non-toxic AF coating was used whose efficacy depend on the low adhesion strength and a low energy is required to remove the fouling organism from the substrate. Author concludes that research is increased to produce coatings with Foul release ability particularly with the development of fluor based polymers and hybrid foul release coatings.

(bers et al., 2004) this paper is about the study of surface microtopography of various marine organisms which is less than 500 μm . scanning electron microscopy was used to characterize the surface topography of all marine organism. Field studies were performed using a high-resolution resin of these marine natural structures to determine the influence of different microtopographies on fouling rates.

(Sampathkumaran et al., 2008) this paper is based on the use of electro chlorination system a method to prevent biofouling in seawater. Biofouling in pipe lets can be prevented using chemical treatment by converting sodium chloride to sodium hypochlorite. Chlorine is the frequently used disinfectant to prevent fouling.

(Legg et al., 2015) this review is about the use of acoustic techniques to inhibit biofouling, mainly using a low ultrasonic frequency. Many laboratory and sea trial studies have reported a successful prevention of biofouling.

(Wang et al., 2020) their work is on the cleaning of fouling organism from the marine substratum by using scraping method. This method is proposed on the principle of orthogonal cutting theory and the peel zone method. Removing of barnacles depend on the blade rake angle. Therefore, a reduction in the cutting force can be achieved by increasing the blade rake angle.

(Burgess et al., 2003) their research is to produce a non-toxic antifouling paint. They have extracted some metabolites from marine epiphytic bacteria isolated from marine environment. These metabolites were incorporated into paint resin and exposed to field studies.

(Dahms H. U and Hellio C, 2009) this literature is on the laboratory bioassays for the screening of activity and toxicity of potential antifouling compounds. Various methods are explained in this paper from bioassay for microbial fouling to macro fouling.

(Nir and Reches, 2016) their review is on the development of non-toxic antifouling approaches. Their main focus is on the latest advances in the field of antifouling materials. The advanced techniques which are discussed here are Surface

topography to mimic the surface topography of marine organisms or plants, DOPA-based antifouling materials an adhesive protein of marine mussels can be used as bio adhesive coating and Quorum-sensing inhibitory compounds as antifouling materials to inhibit the bacterial communication.

(Manilal et al., 2010) their research was on the antifouling potentials of seaweed against marine fouling organisms. The red sea algae showed a broad range of activity, which was further, purified by column chromatography and identified for specific compound using GC-MS.

CHAPTER 3

3. AIM AND SCOPE:

- To prepare a green emulsion containing plant extract with commercial enamel paint.
- To prepare the various antifouling substrates and their investigation studies in Vellar estuary.
- To evaluate the antifouling activity of extracted sample against the fouling organisms.
- To determine the minimum inhibitory concentration of plant extract.
- To study the functional group of the crude plant extracts using FTIR.

CHAPTER 4

4. MATERIALS AND METHODS:

Methanolic extracts of three salt marsh plants were tested against marine fouling bacteria. These extracts were also incorporated into the paints for field trials.

4.1 Materials:

1. Solvent: Methanol
2. Plant sample:
 - Sample I – *Ipomoea pes-caprae*
 - Sample II – *Sesuvium portulacastrum*
 - Sample III – *Batis maritima*
3. Wares: Mortar and pestle, Conical flask, Funnel, Measuring cylinder, Petri plates, Whatman filter paper no: 1, Aluminum foil paper, Micropipettes, Pipette tips, Cotton swab, Test tubes, Test tube stand, Bunsen burner, Nylon rope, Asian enamel paint 500ml (blue), Thinner 200ml, Paint brush.
4. Instruments: Shaker (Remi Limited), Rotary evaporator (Cyber lab), Laminar air flow chamber, lyophilizer (Delvac), FTIR (Shimadzu), Autoclave, UV spectroscopy (Molecular Devices).
5. Substrates
 - Wood
 - Aluminum sheet
 - Fiber sheet
6. Media:
 1. Mueller Hinton agar:
 - Mueller Hinton agar - 32 g/L
 - Agar - 10 g/L
 2. Mueller Hinton broth:
 - Mueller Hinton broth – 38g/L
 3. Nutrient broth:
 - Nutrient broth – 13g/L
 4. Zobell agar
 - Zobell agar – 55.25g/L
 - Agar – 10g/L

4.2 Sample collection:

Three salt marsh plants were collected from southeast coast of India. *Ipomoea pes-caprae* and *Sesuvium portulacastrum* from the coastline of Singarathoppu. *Batis maritima* from the coast area of Parangipettai.

4.3 Solvent Extraction:

Solvent extraction was carried out as previously reported (khan et al., 2014) with appropriate modification. The plant leaves were washed with sea water subsequently with tap water to remove the dirt. The washed leaves were pat dried to remove the moisture content. 100g of fresh leaves were weighed and crushed using mortar and pestle. The crushed samples were soaked in 400mL of methanol (1: 4 ratios) and kept in the shaker (30^{0c}) for 36 hours at 100 rpm. The plant extracts were filled through Whatman No.1 filter papers. The extracts were condensed by using rotary evaporator and lyophilized. The obtained condensed extracts were stored for further assay.

4.4 Field trials:

4.4.1 Preparation of Emulsion:

Enamel paint were chosen for the study because it is commonly used paint to coat the submerged structures. In this study, Asian enamel paint was incorporated with different types of solvents and samples at the ratio of 4:1(40mL+10mL). Five separate beakers were taken to check the solubility of the paint namely,

- Beaker A - paint + thinner
- Beaker B - paint + solvent(methanol)
- Beaker C - paint + *Ipomoea pes-caprae* extract
- Beaker D - paint + *Sesuvium portulacastrum* extract
- Beaker E - paint + *Batis maritima* extract

4.4.2 Preparation of raw materials:

The raw materials were used are Wood, Aluminium and Fibre sheets. These materials were cut in the dimension of 6*6 inches. The prepared paint was double coated on the raw materials using paint brush. The paint coated raw materials were shade dried for 10 days.

4.4.3 Study area:

The dried substrates were tied using a nylon rope at vellar estuary, opposite to marine biology station, parangipettai (11⁰29'N, 79⁰46'E). Substrates were partially submerged at lowest low tide region. The tidal range in this zone is 1m with the

average depth of 2.5m. Nature of the substratum is sandy-muddy which comprises less than 20% slit and higher clay content with the salinity range of 28.13 ppt (ENVIS).

4.5 Antifouling assays:

4.5.1 Microorganisms and Inoculum development:

The crude methanolic extracts were test against the bacterial pathogens namely *Pseudomonas sp.*, *Vibrio sp.*, *Proteus vulgaris*, *Proteus mirabilis*, *E. coli* and *staphylococcus aureus*. All microorganisms were collected from medical college, Annamalai University, Chidambaram.

The bacterial strains were subcultured in 5ml of nutrient broth. The strains were inoculated in marine zobell agar (HiMedia) to observe the single bacterial colonies after 24 h.

4.5.2 Antifouling activity:

Antimicrofouling activity using methanolic extract was performed as previously described (S. Satheesh et al., 2012) with right changes. Antibacterial activity was carried out using disc diffusion method against six biofouling bacteria. Mueller Hinton agar (HiMedia) was prepared, sterilized and 20ml of media was poured to each sterile Petri plates aseptically. After solidification, 10µl of bacterial culture was swabbed using a cotton swab onto the agar medium and allowed to dry for 5 minutes. The disc was made with Whatman No: 1 filter paper with 6mm in diameter. These discs were impregnated in the Petri plates. Different concentration of crude extracts of 100µl, 150µl and 200µl were loaded onto the disc. Tetracycline was used as a control. The plates were incubated at 37⁰c for 24 h. After incubation, zone of inhibition was observed around the disc and the zone size was measured in millimeters.

4.5.3 Minimum inhibitory concentration (MIC):

Minimum inhibitory concentration was carried as described (Murakami et al., 2019). Minimum inhibitory concentration (MIC) was used to determine the lowest concentration of the sample required to inhibit the growth of the bacterial strains. MIC for different bacterial strains was performed using different concentrations. The sterile test tubes were filled with 5ml of Mueller Hinton broth (HiMedia) and different concentration of crude extracts (100 µg/mL, 200 µg/mL and 300µg/mL) was added. These test tubes were incubated with 10µL of bacterial inoculum from 24 h old culture. The turbidity was observed after 24 h using UV-spectroscopy at

600nm and the results were tabulated.

4.6 FTIR spectral analysis:

The identification of functional groups of the sample was obtained by using Fourier transform infrared spectroscopy. The study uses an infrared radiation on the sample which causes an atomic vibration of a molecule present in the sample, resulting in a particular absorption or transmission of energy. FTIR is used to determine the chemical bond and the organic content present in the sample (Nandiyanto, A. B. D. et al., 2019). Sample preparation for FTIR analysis was carried out using known concentration of the extract. A small drop of sample was placed on KBr plate and using second plate a thin even film was made. Plates were placed into the sample holder and run a spectrum.

CHAPTER 5

5. RESULT AND DISSCUSION:

5.1 Sample collection:



Fig 5.1 Ipomoea pes-caprae



Fig 5.2 Sesuvium portulacastrum



Fig 5.3 Batis maritima

5.2 Sample preparation:



Fig 5.4 Samples were crushed by using motor and pestle method

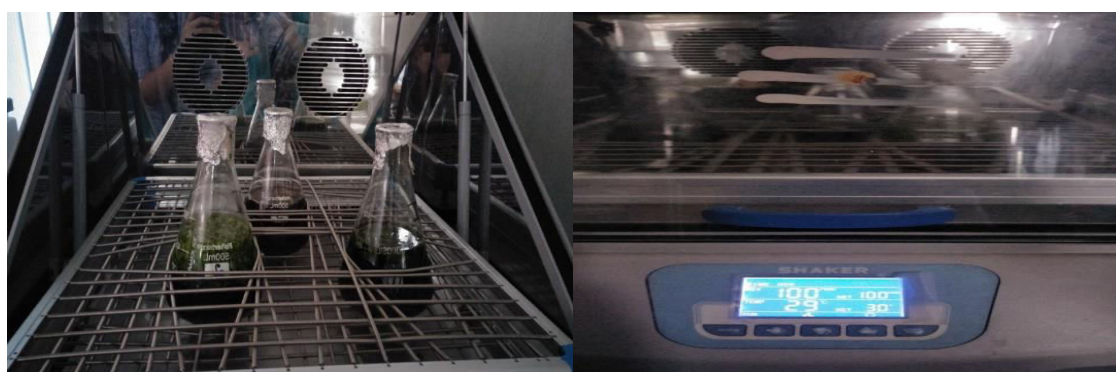


Fig 5.5 Crushed samples kept in Shaker for incubation

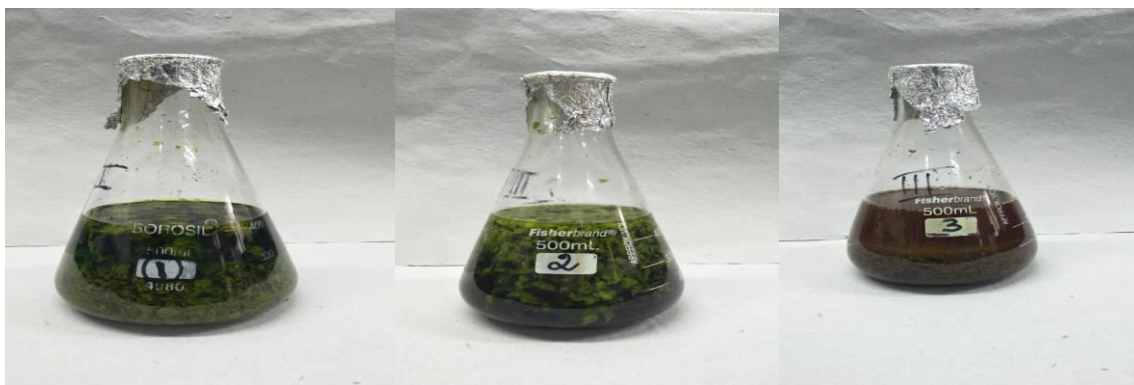


Fig 5.6 Samples after incubation

5.3 Emulsion preparation:



Fig 5.7 Asian enamel paint and Thinner

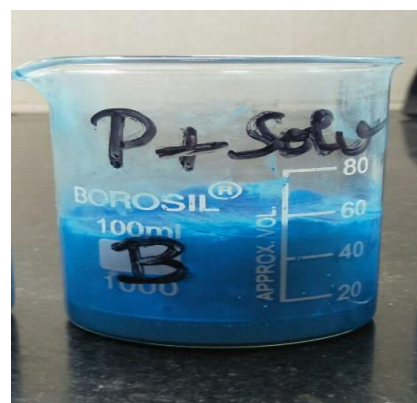


Fig 5.9 Paint + Solvent

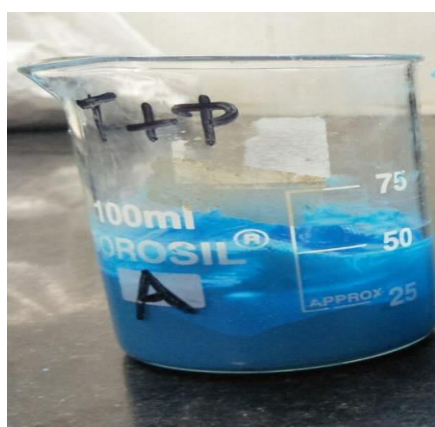


Fig 5.8 Paint + Thinner

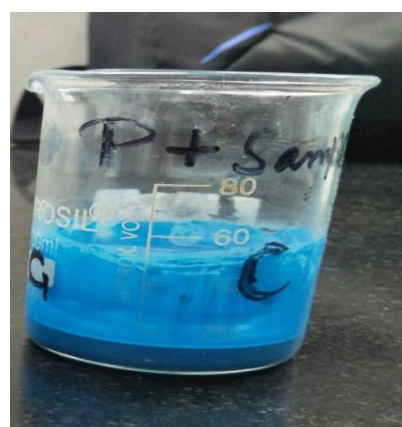


Fig 5.10 Paint + Sample I



Fig 5.11 Paint +sample II



Fig 5.12 Paint + sample III

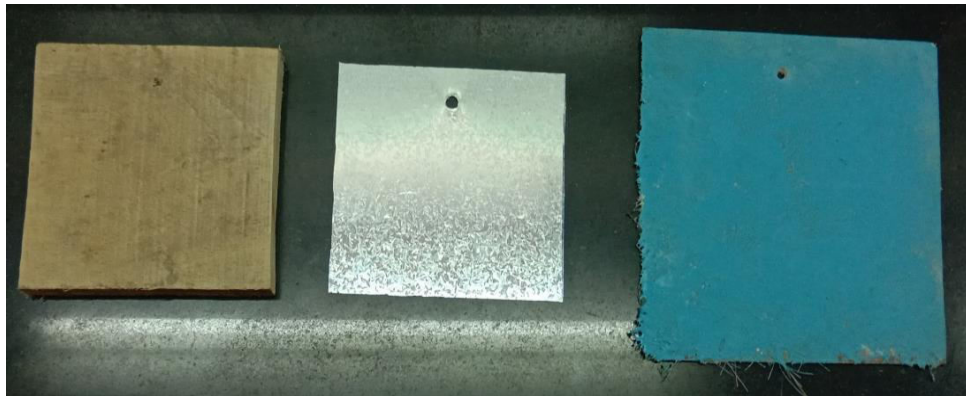


Fig 5.13 Raw materials



Fig 5.14 Controls (uncoated substrates)

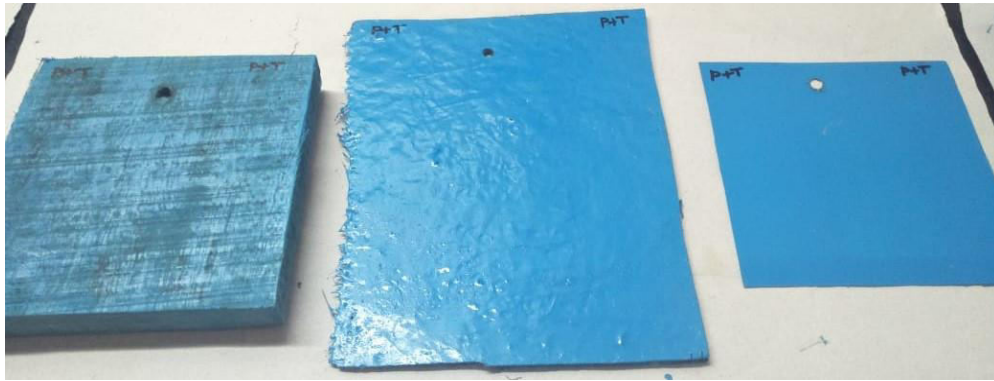


Fig 5.15 Paint and thinner coated substrates

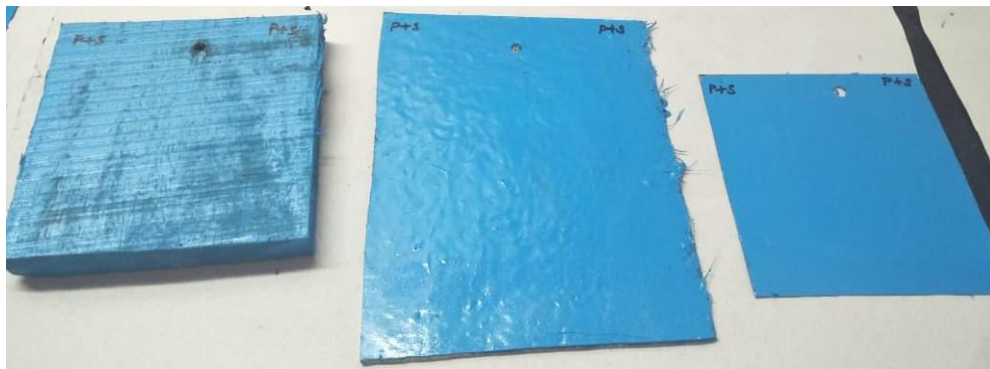


Fig 5.16 Paint and solvent coated substrates

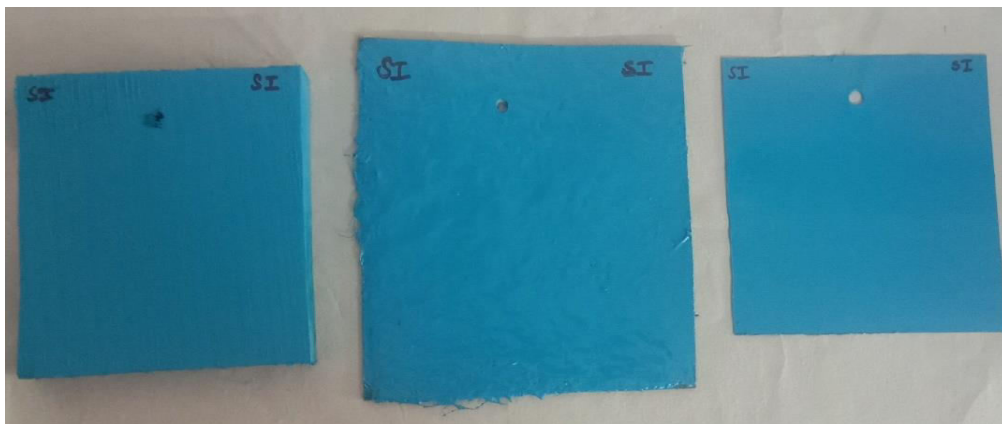


Fig 5.17 Paint and sample I coated substrates



Fig 5.18 Paint and sample II coated substrates

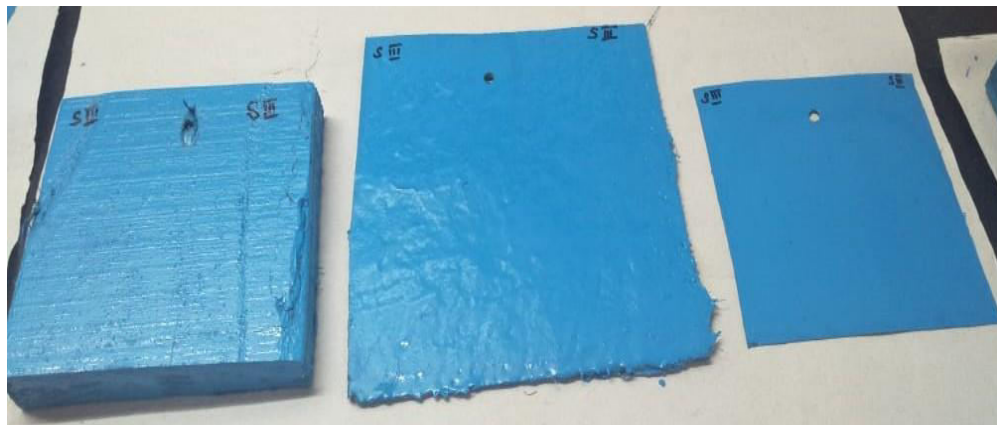


Fig 5.19 Paint and sample III coated substrates



Fig 5.20 The full picture of substrates before immersing at estuary



Fig 5.21 The tied substrates immersed at lowest low tide of the estuary



Fig 5.22 Location site where the fields study was performed

5.4 Field study results:

5.4.1 After 15 days of observation:

The results of field study after 15 days of observation indicates that only in control, solvent and paint coated substrates, thinner and paint coated substrates have some foul formation. Whereas the sample and paint coated substrates, they have less or 0% of foul formation.

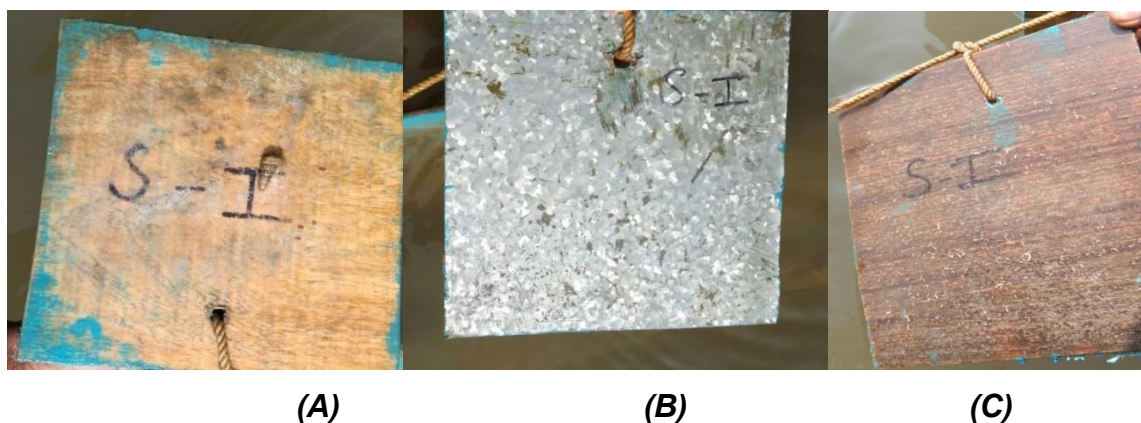


Fig 5.23 Uncoated substrates kept for control (A) wood, (B) Aluminum sheet, (C) Fibre sheet.

In the fig 5.23 the uncoated substrates (control) represent the foul formation on wood was 1%, aluminum 0% and fiber 5% which is observed after 15 days.

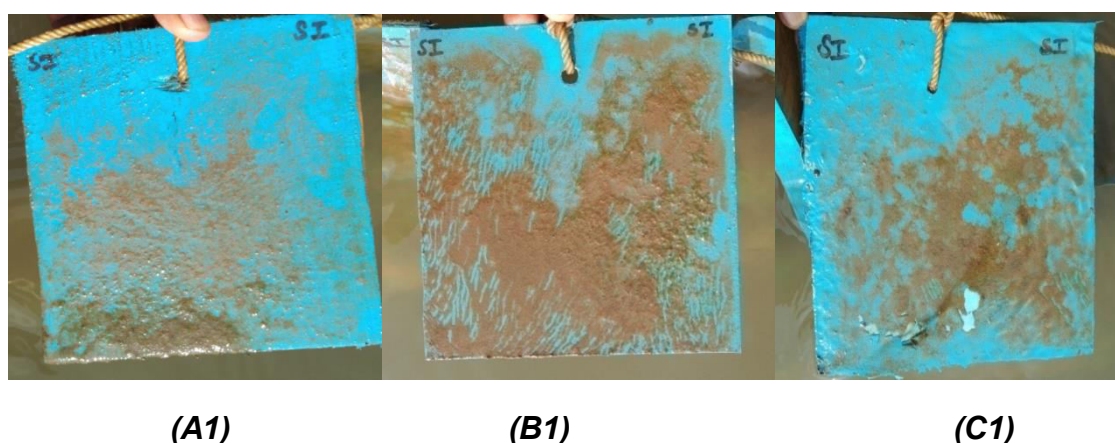
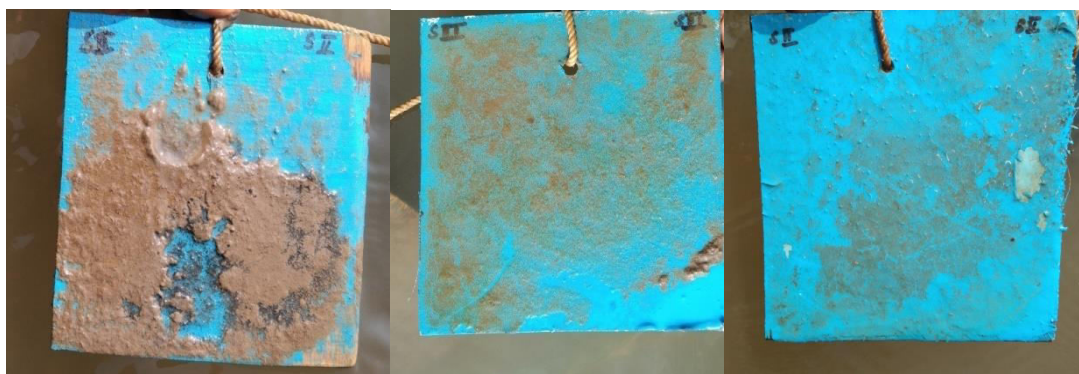


Fig 5.24 The sample I and paint coated substrates (A1) sample I + paint coated wood, (B1) sample I + paint coated aluminium sheet and (C1) sample I + paint coated fibre sheet.

The above figure 5.24 represents the substrates coated by methanolic extract of *Ipomoea pes-caprae* mixed with paint and the foul formation after 15 days on wood was 0%, aluminium sheet was 0% and fiber was 1%.



(A2)

(B2)

(C2)

Fig 5.25 The sample II and paint coated substrates (A2) sample II + paint coated wood, (B2) sample II + paint coated aluminum sheet and (C2) sample II + paint coated fibre sheet.

The above fig 5.25 represents the substrates coated by methanolic extract of *Sesuvium portulacastrum* mixed with paint and the foul formation after 15 days on wood was 0%, aluminium sheet was 0% and fiber was 1%.



(A3)

(B3)

(C3)

Fig 5.26 Sample III + paint coated substrates (A3) sample III+ paint coated wood, (B3) sample III+ paint coated aluminium sheet and (C3) 3 + paint coated fibre sheet.

The above fig 5.26 represents the substrates coated by methanolic extract of *Batis maritima* mixed with paint and the foul formation after 15 days on wood was 0%, aluminium sheet was 0% and fiber was 1%.



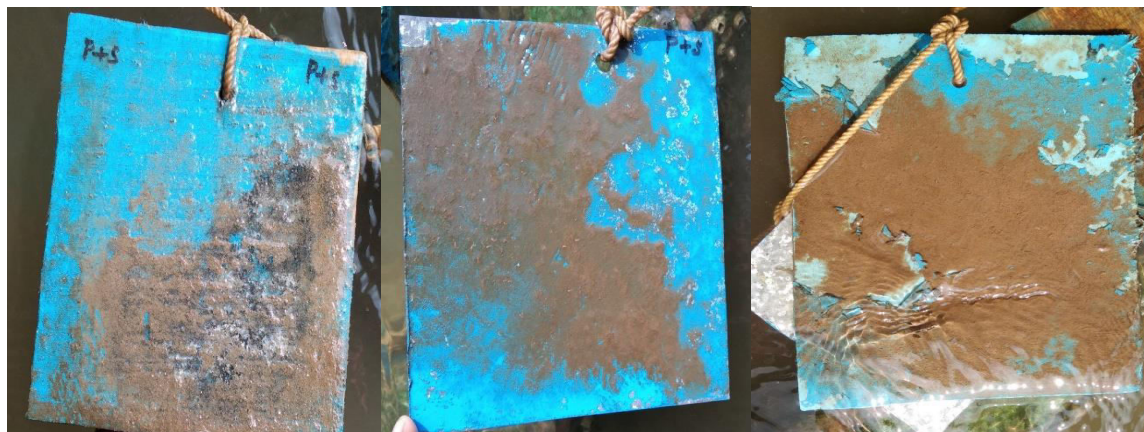
(A4)

(B4)

(C4)

Figs 5.27 Thinner + paint coated substrates (A4) thinner + paint coated wood, (B4) thinner + paint coated aluminum sheet and (C4) thinner + paint coated fibre sheet

The above fig 5.27 represents the substrates coated by thinner mixed with paint and the foul formation after 15 days on wood was 2%, aluminium sheet was 0% and fiber was 2%.



(A5)

(B5)

(C5)

Fig 5.28 Solvent + paint coated substrates (A5) Solvent + paint coated wood, (B4) Solvent + paint coated aluminium sheet and (C4) Solvent + paint coated fibre sheet.

The above fig 5.28 represents the substrate coated by solvent mixed with paint and the foul formation after 15 days on wood was 3%, aluminium 4% and fibre 6%.

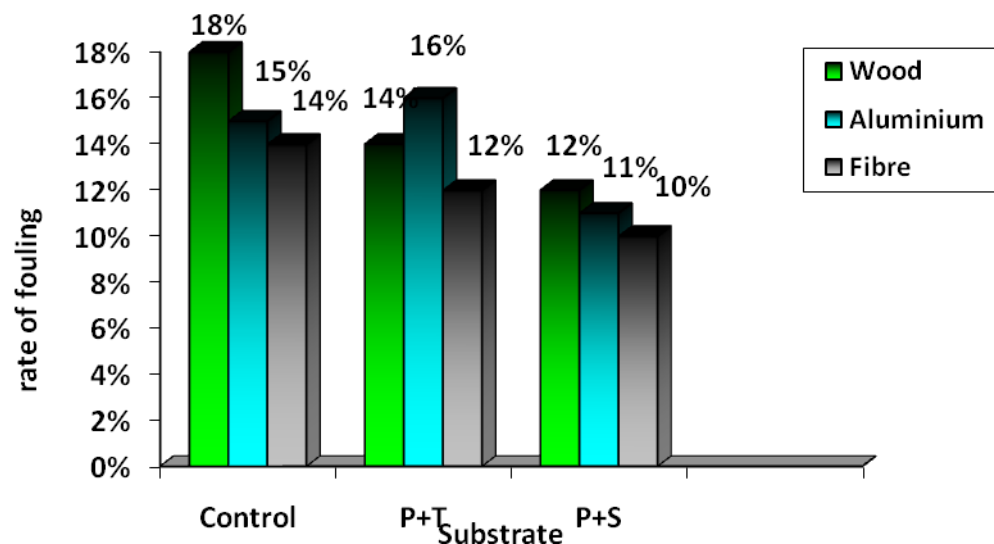


Fig 5.29 Graph represents the foul formation on wood, aluminium and fibre in percentage after 15 days of observation

The above graph shows that control (uncoated substrate) has more foul formation when compared to paint + thinner and Paint + Solvent.

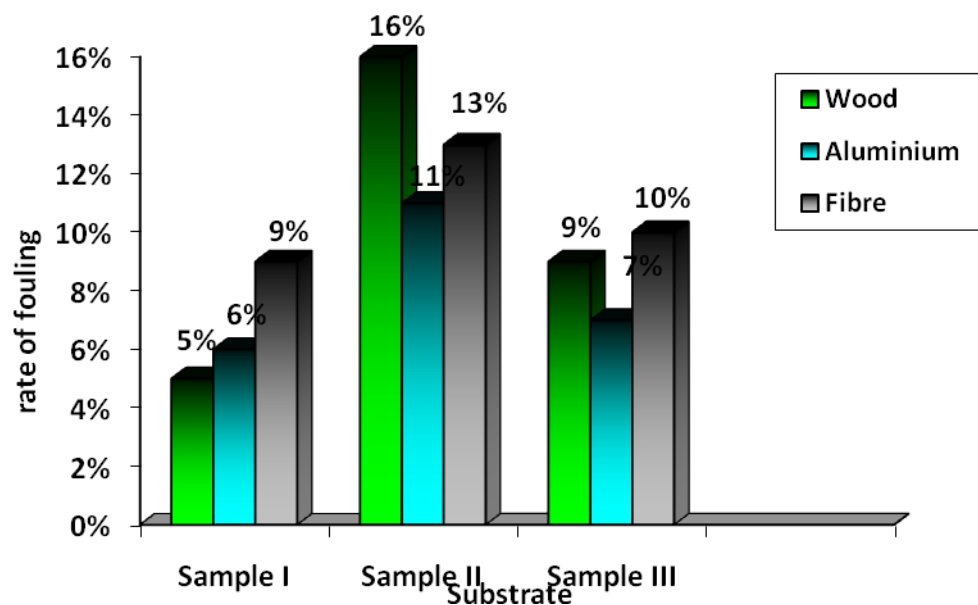


Fig 5.30 Graph represents the foul formation on different substrates in percentages after 15 days of observation

The above graph shows that sample I have less foul formation followed by sample III and sample II.

5.4.2 After observation of 31 days:

The results of field study after 31 days of observation shows that control has more foul formation than the other coated substrates and among all the substrates foul formation on wood (sample I + paint) gives the best result.

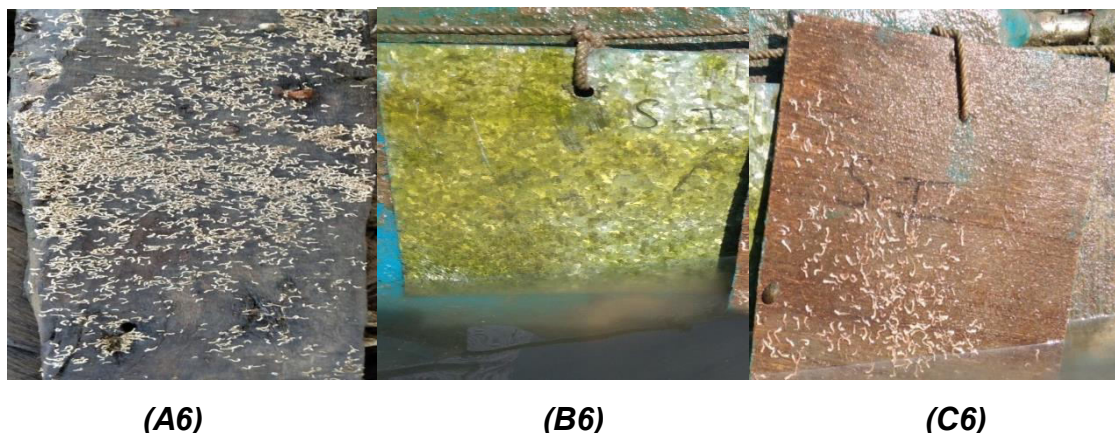


Fig 5.31 The uncoated control (A6) wood, (B6) aluminium sheet and (C6) fibre sheet

The above fig 5.31 represents the uncoated substrates (control) and the foul formation after 31 days on wood was 98%, aluminium sheet was 85% and fiber was 65%.

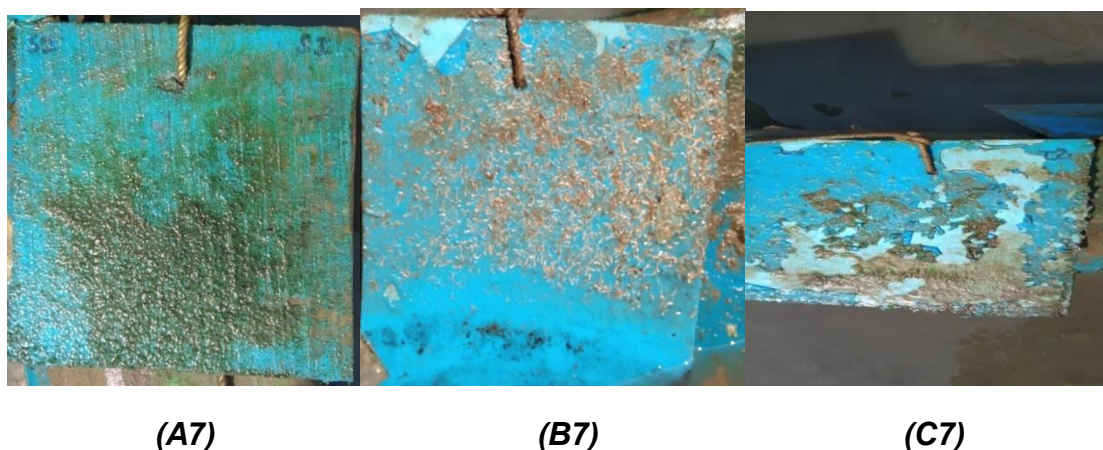


Fig 5.32 Sample I + paint coated substrates, (A7) sample I + paint coated wood, (B7) sample I + paint coated aluminium sheet and (C7) sample I + paint coated fibre sheet.

The above fig 5.32 represents the substrates coated by methanolic extract of *Ipomoea pes-caprae* mixed with paint and the foul formation after 15 days on wood was 0%, aluminium sheet was 0% and fiber was 1%.



(A8)

(B8)

(C8)

Fig 5.33 Sample II + paint coated substrates, (A8) sample II + paint coated wood, (B8) sample II + paint coated aluminium sheet and (C8) sample II + paint coated fibre sheet.

The above fig 5.33 represents the substrates coated by methanolic extract of *Sesuvium portulacastrum* mixed with paint and the foul formation after 31 days on wood was 17%, aluminium sheet was 68% and fiber was 75%.



(A9)

(B9)

(C9)

Fig 5.34 Sample III + paint coated substrates, (A9) sample III+ paint coated wood, (B9) sample III + paint coated aluminium sheet and (C9) sample III + paint coated fibre sheet .

The above fig 5.34 represents the substrates coated by methanolic extract of *Batis maritima* mixed with paint and the foul formation after 31 days on wood was 47%, aluminium sheet was 42 % and fiber was 89%.



Fig 5.35 Solvent + paint coated substrates wood, aluminium sheet and fibre sheet.

The above fig 5.35 represents the substrates coated by solvent with paint and the foul formation after 31 days on wood was 42%, aluminium sheet was 36% and fiber was 57%.

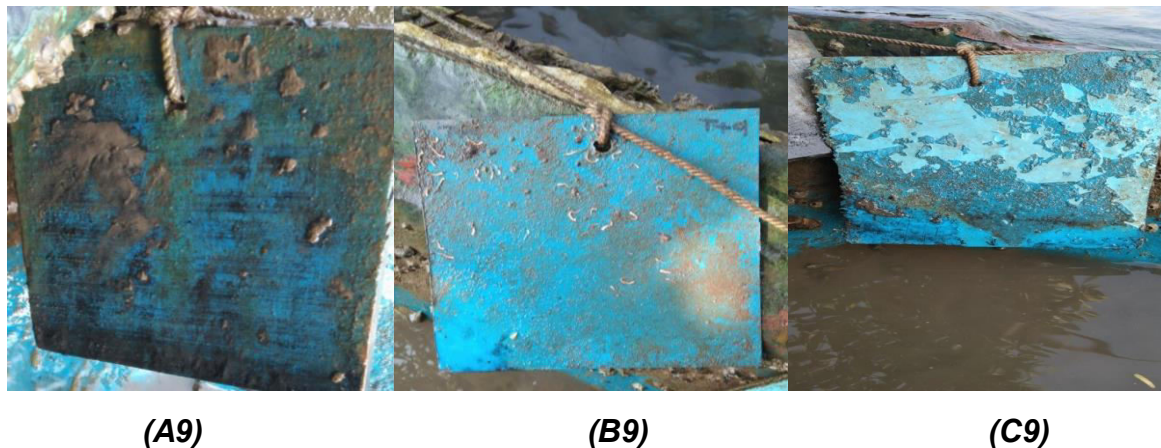


Fig 5.36 Thinner + paint coated substrates, (A9) thinner + paint coated wood, (B9) thinner + paint coated aluminium sheet and (C9) thinner + paint coated fibre sheet.

The above fig 5.36 represents the substrates coated by thinner with paint and the foul formation after 31 days on wood was 35%, aluminium sheet was 59% and fiber was 80%.

From the field study fig 5.32 shows better result for paint + sample I coated wood

when compared to control the uncoated substrates (fig 5.31) shows fouling formation. Hence sample I show better activity for 31 days of observation. Therefore, salt marsh plant has activity against marine fouling organisms. Sample II and sample III shows moderate activity.

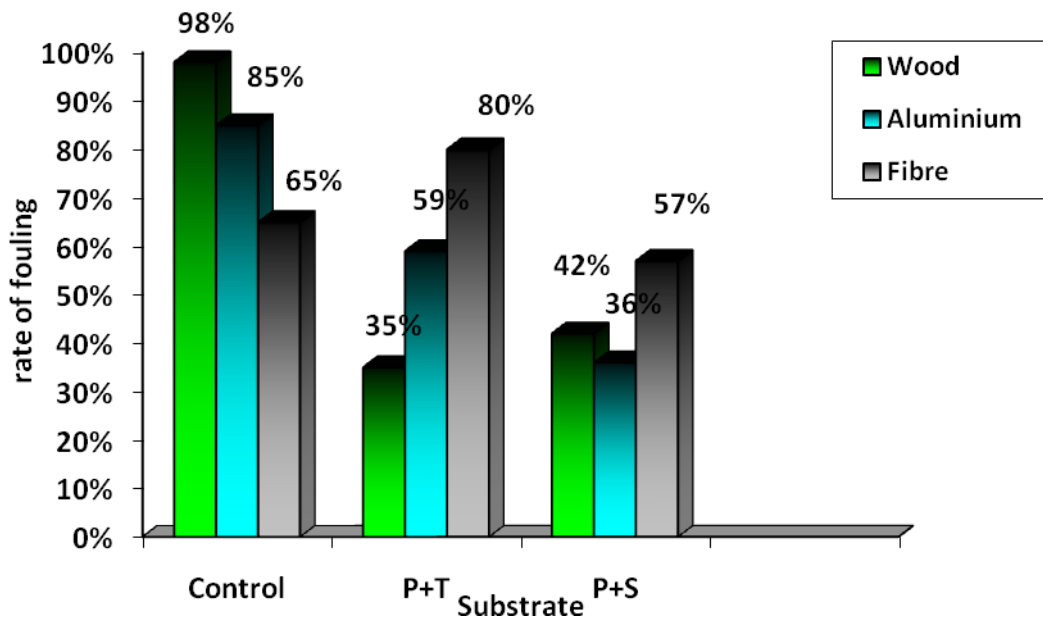


Fig 5.37 Graph represents the foul formation on different substrates in percentage after 31 days of observation.

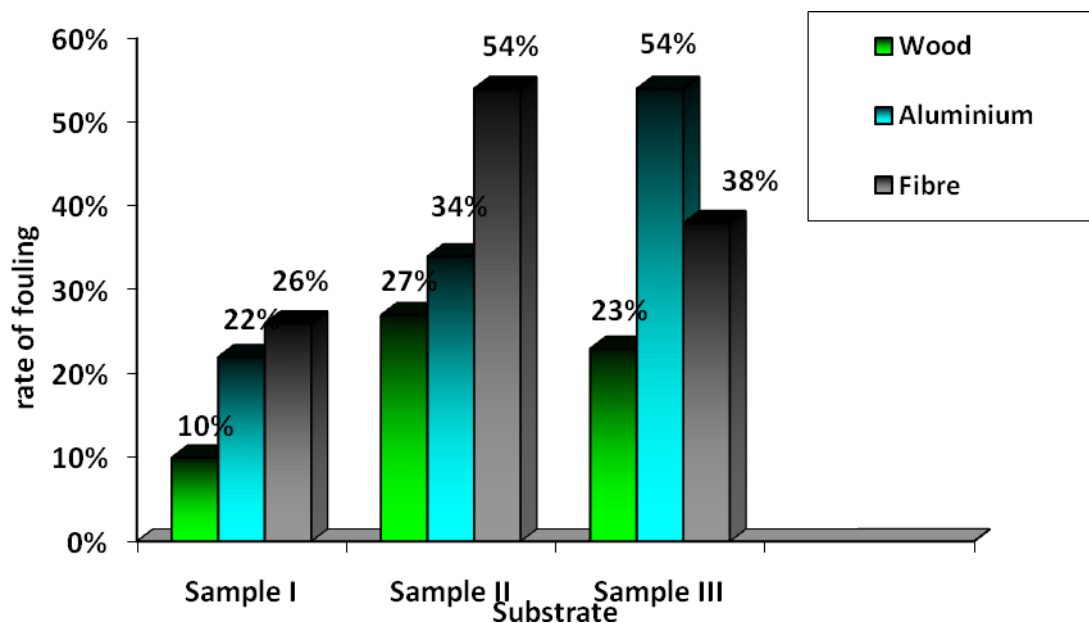


Fig 5.38 Graph represents the foul formation on different substrates in percentage after 31 days of observation.

The above graph shows that sample I have less foul formation followed by sample II and sample III. Therefore, sample I was very effective against fouling organisms.

5.5 Antifouling activity against marine fouling organism at various concentrations by disc diffusion methods:

Antifouling assay has done against six organisms using three samples at 100 μ l, 150 μ l and 200 μ l concentrations. In that methanolic extracts of *Ipomoea pes-caprae* forms the zone of inhibition up to 26 mm at 200 μ l concentration and the control used here is antibiotic tetracycline to compare the activity of the samples.



Fig 5.39 The plate without culture swab (control)



(A10)

(B10)

(C10)

Fig 5.40 Zone of inhibition formed at various concentrations against *Porteus vulgaris*, (A10) sample I, (B10) sample II and (C10) sample III

The methanolic extract (10mg/mL) of all sample showed more significant

antibacterial activity against *Proteus vulgaris*. In figure 5.40, for *Ipomoea pes-caprae* (A10) the zone of inhibition obtained at 100 μ L was 13mm, at 150 μ L was 24mm, at 200 μ L was 25 mm and control (14mm). For *Sesuvium portulacastrum* (B10) the zone of inhibition obtained at 100 μ L was 10mm, at 150 μ L was 12mm, at 200 μ L was 13mm and control (12mm). For *Batis maritima* (C10) the zone of inhibition obtained at 100 μ L was 10mm, at 150 μ L was 12mm, at 200 μ L was 14mm and control (12mm). From above result, *Ipomoea pes-caprae* showed a maximum zone of inhibition against *Proteus vulgaris* at 200 μ L (25mm).

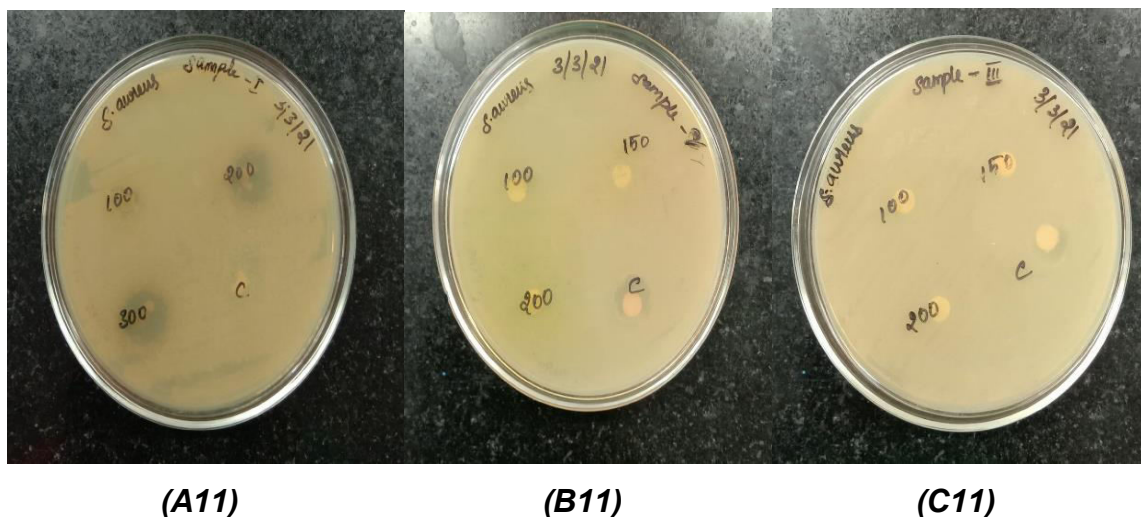


Fig 5.41 Zone of inhibition formed at various concentrations against *Staphylococcus aureus*, (A11) sample I, (B11) sample II and (C11) sample III

In figure 5.41, for *Ipomoea pes-caprae* (A11) the zone of inhibition obtained at 100 μ L was 9mm, at 150 μ L was 11mm, at 200 μ L was 13mm and control (10mm). For *Sesuvium portulacastrum* (B11) the zone of inhibition obtained at 200 μ L was 13mm, control (12mm) and no zone formation at 200 μ L and 150 μ L. For *Batis maritima* C11 the zone of inhibition obtained at 150 μ L was 9mm, at 200 μ L was 13mm, control (11mm) and no zone formation at 100 μ L.

From above result, *Ipomoea pes-caprae* and *Batis maritima* showed a maximum zone of inhibition against *staphylococcus aureus* at 200 μ L (13mm).



(A12)

(B12)

(C12)

Fig 5.42 Zone of inhibition formed at various concentrations against *Escherichia coli*, (A12) sample I, (B12) sample II and (C12) sample III.

In figure 5.42, for *Ipomoea pes-caprae* (A12) the zone of inhibition obtained at 100 μ L was 18mm, at 150 μ L was 20mm, at 200 μ L was 24mm and control (14mm). For *Sesuvium portulacastrum* (B12) there was no zone formation and control (14mm). For *Batis maritima* (C12) the zone of inhibition obtained at 100 μ L was 11mm, at 150 μ L was 12mm, at 200 μ L was 14mm and control (11mm).

From above result, *Ipomoea pes-caprae* showed a maximum zone of inhibition against *Escherichia coli* at 200 μ L (24mm).



(A13)

(B13)

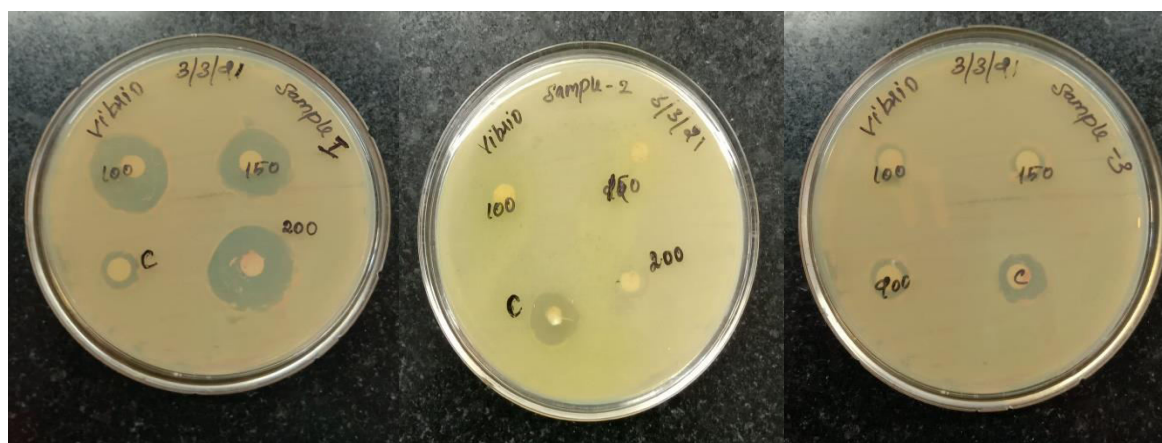
(C13)

Fig 5.43 Zone of inhibition formed at various concentrations against *Porteus mirabilis* (A13) sample I, (B13) sample II and (C13) sample III

In figure 5.43, for *Ipomoea pes-caprae* (A13) the zone of inhibition obtained at 100 μ L was 13mm, at 150 μ L was 15mm, at 200 μ L was 19 mm and control (12mm).

For *Sesuvium portulacastrum* (B13) the zone of inhibition obtained at 100 μ L was 9mm, at 150 μ L was 10mm, at 200 μ L was 12mm and control (11mm). For *Batis maritima* (C13) there was no zone formation and control (12mm).

From above result, *Ipomoea pes-caprae* showed a maximum zone of inhibition against *Proteus mirabilis* at 200 μ L (19mm).



(A14)

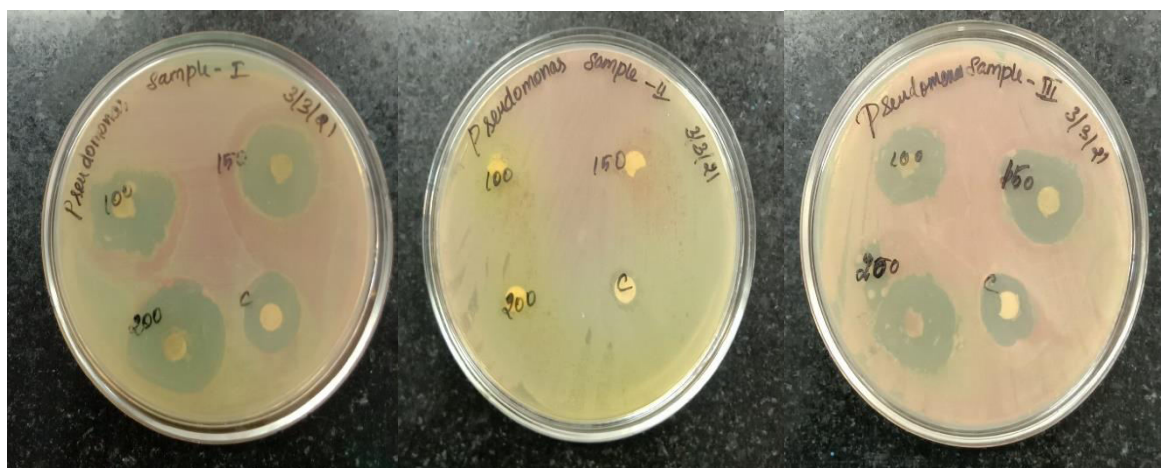
(B14)

(C14)

Fig 5.44 Zone of inhibition formed at various concentrations against *Vibrio* sp. (A14) sample I, (B14) sample II and (C14) sample III

In figure 5.44, for *Ipomoea pes-caprae* (A14) the zone of inhibition obtained at 100 μ L was 20mm, at 150 μ L was 19mm, at 200 μ L was 23 mm and control (9mm). For *Sesuvium portulacastrum* (B14) there was no zone formation and control (13mm). For *Batis maritima* (C14) the zone of inhibition obtained at 100 μ L was 8mm, at 150 μ L was 10mm, at 200 μ L was 11mm and control (13mm).

From above result, *Ipomoea pes-caprae* showed a maximum zone of inhibition against *Vibrio* sp. at 200 μ L (23mm).



(A15)

(B15)

(C15)

Fig 5.45 Zone of inhibition formed at various concentration against *Pseudomonas* sp. (A15) sample I, (B15) sample II and (C15) sample III

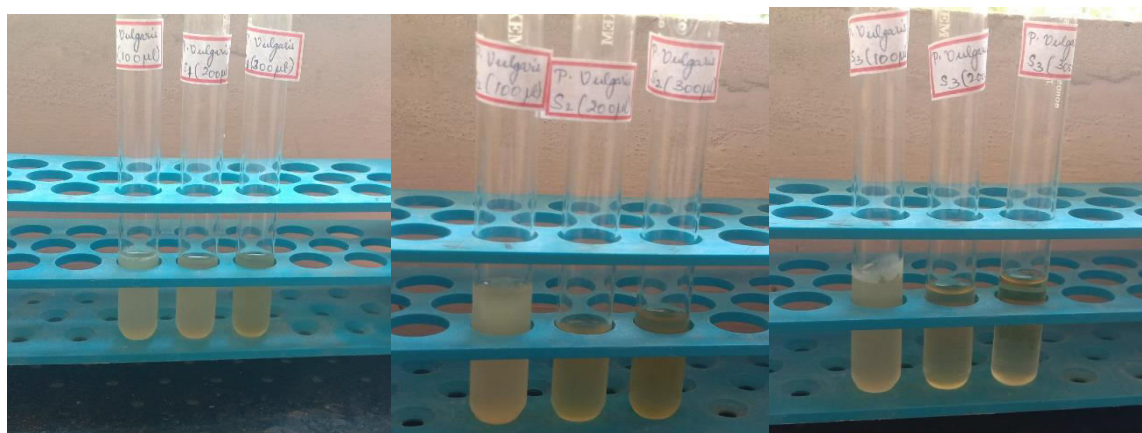
In figure 5.45, for *Ipomoea pes-caprae* (A15) the zone of inhibition obtained at 100 µL was 21mm, at 150 µL was 22mm, at 200 µL was 26 mm and control (16mm). For *Sesuvium portulacastrum* (B15) there is no zone formation and control (9mm). For *Batis maritima* (C15) the zone of inhibition obtained at 100 µL was 18mm, at 150 µL was 21mm, at 200 µL was 23mm and control (15mm).

From above result, *Ipomoea pes-caprae* showed a maximum zone of inhibition against *Pseudomonas* sp. at 200 µL (26mm).

Table 5.1 Indicates zone of inhibition formed at various concentrations against six Marine fouling organisms and Tetracycline at 50 µl (Antibiotic) was given as control to compare the activity of the sample

Species	Methanolic extract of <i>Ipomoea pes-caprae</i> (10mg/mL) at mm				Methanolic extract of <i>Sesuvium portulacastrum</i> (10mg/mL) at mm				Methanolic extract of <i>Batis maritima</i> (10mg/mL) at mm			
	100 µl	150 µl	200 µl	C µl	100 µl	150 µl	200 µl	C µl	100 µl	150 µl	200 µl	C µl
<i>Escherichia coli</i>	18	20	24	14	-	-	-	14	11	12	14	11
<i>Proteus mirabilis</i>	13	15	19	12	9	10	12	11	-	-	-	13
<i>Proteus vulgaris</i>	13	24	25	14	10	12	13	12	10	12	14	12
<i>Pseudomonas sp.</i>	21	22	26	16	-	-	-	9	18	21	23	15
<i>Staphylococcus aureus</i>	9	11	13	10	-	-	13	16	-	9	13	11
<i>Vibrio sp.</i>	20	19	23	9	-	-	-	13	8	10	11	13

5.6 Minimum Inhibitory Concentration against marine fouling bacteria at various concentrations:



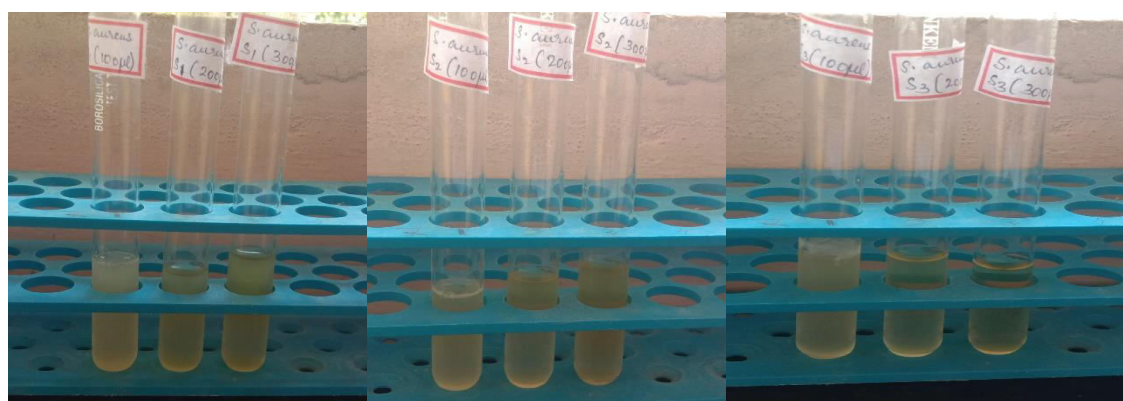
(A16)

(B16)

(C16)

Fig 5.46 Turbidity level at different concentration against *Proteus Vulgaris* (A16) sample I, (B16) sample II, (C16) sample III.

From the above figure(5.46) less turbidity was observed in *Ipomoea pes-caprae* (A16) against *Proteus vulgaris* at 300 μ L (10mg/mL) compared to other samples.



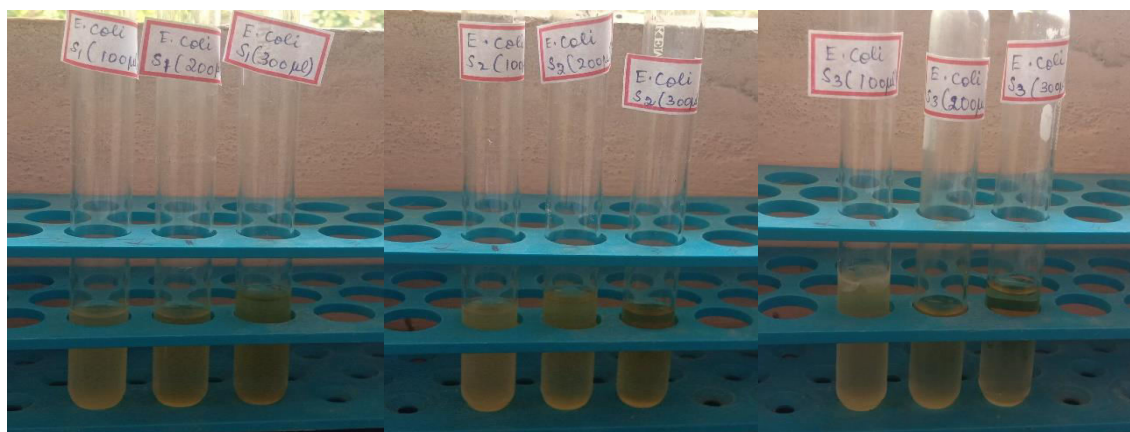
(A17)

(B17)

(C17)

Fig 5.47 Turbidity level at different concentration against *Staphylococcus aureus* (A17) sample I, (B17) sample II, (C17) sample III.

From the above figure(5.47) less turbidity was observed in *Sesuvium portulacastrum* (A17) against *staphylococcus aureus* at 300 μ L (10mg/mL) compared to other samples .



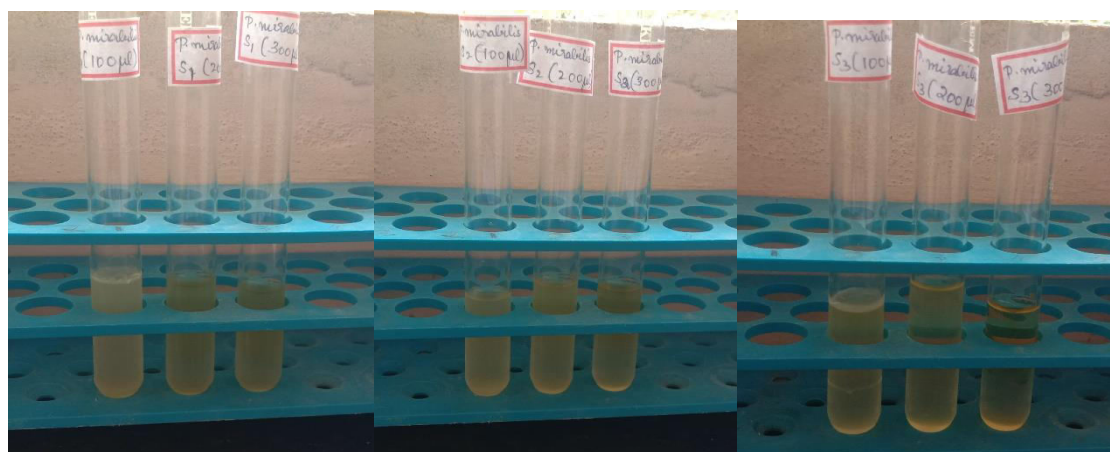
(A18)

(B18)

(C18)

Fig 5.48 Turbidity level at different concentration against *Escherichia coli* (A18) sample I, (B18) sample II, (C18) sample III.

From the above figure(5.48) less turbidity was observed in *Ipomoea pes-caprae* (A18) against *Escherichia coli* at 300 μ L(10mg/mL) compared to other samples .



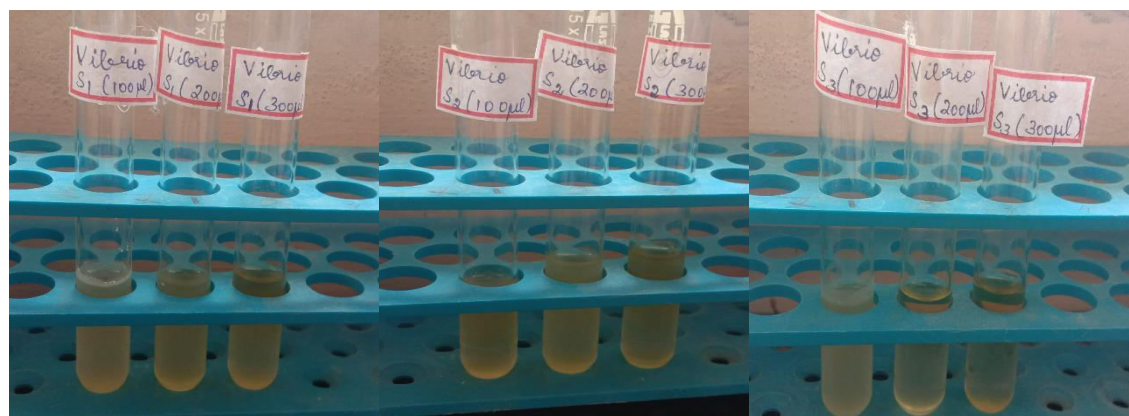
(A19)

(B19)

(C19)

Fig 5.49 Turbidity level at different concentration against *Proteus mirabilis* (A19) sample I, (B19) sample II, (C19) sample III.

From the above figure(5.49) less turbidity was observed in *Ipomoea pes-caprae*(A19) against *Proteus Mirabilis* at 300 μ l(10mg/mL) compared to other samples .



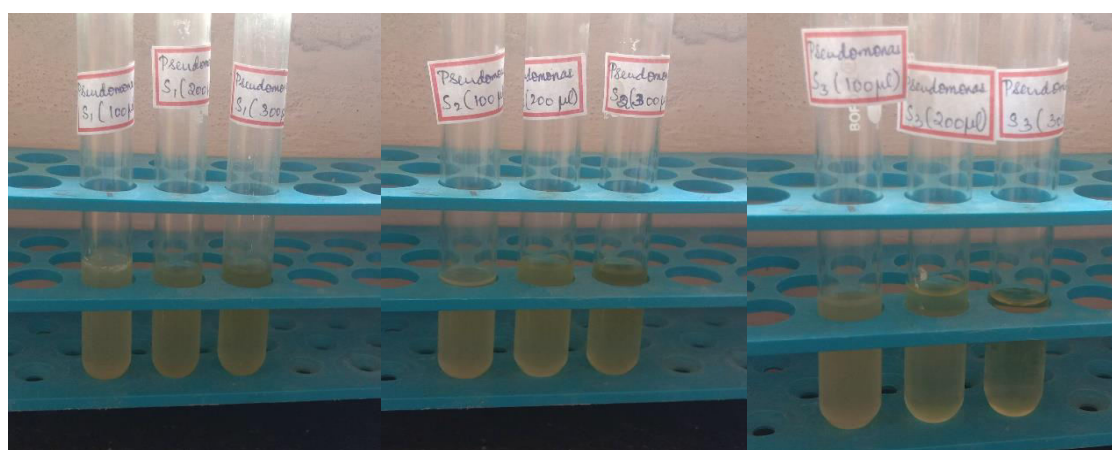
(A20)

(B20)

(C20)

Fig 5.50 Turbidity level at different concentration against *Vibrio* sp. (A20) sample I, (B20) sample II, (C20) sample III.

From the above figure(5.50) less turbidity was observed in *Ipomoea pes-caprae*(A20) against *Vibrio* sp. at 300 µl(10mg/mL) compared to other samples.



(A21)

(B21)

(C21)

Fig 5.51 Turbidity level at different concentration against *Pseudomonas* sp. (A21) sample I, (B21) sample II, (C21) sample III.

From the above figure(5.47) less turbidity was observed in both *Ipomoea pes-caprae*(A20) and *Batis maritima*(C21) against *Pseudomonas* sp. at 300 µL(10mg/mL) compared to other sample.

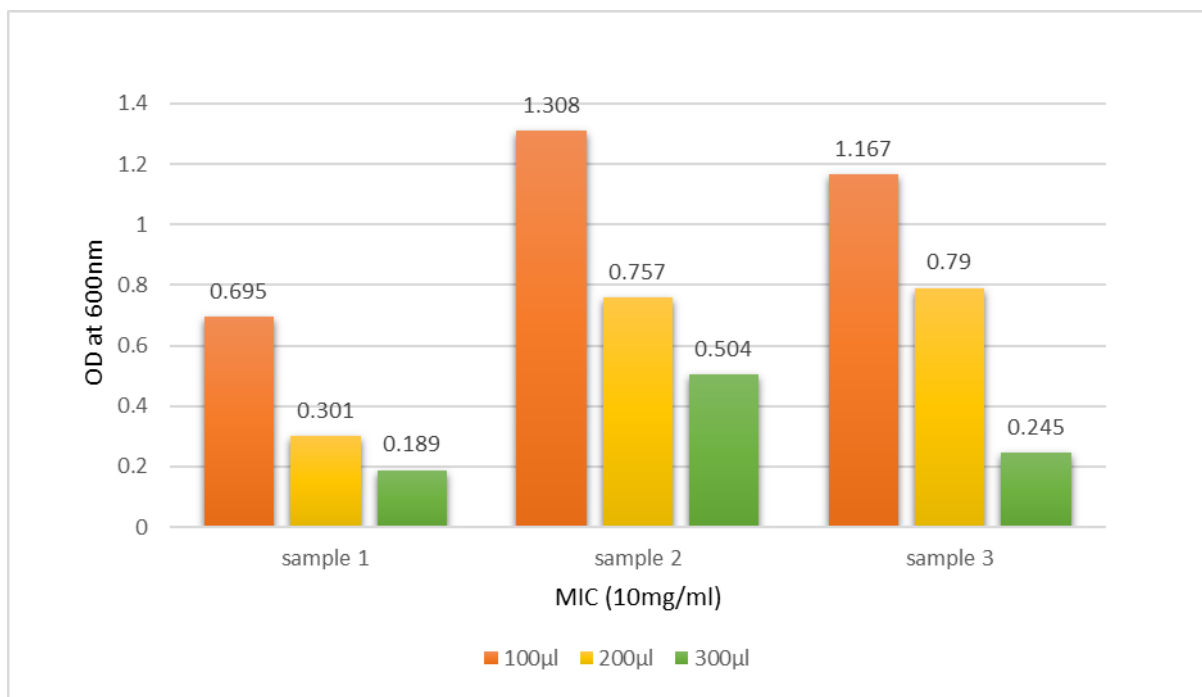


Fig 5.52 Minimum inhibitory concentrations of *Escherichia coli*

The above graph depicts that, there is a steady decrease in the optical density measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* showed a superior activity with OD value 0.189 against *Escherichia coli*.

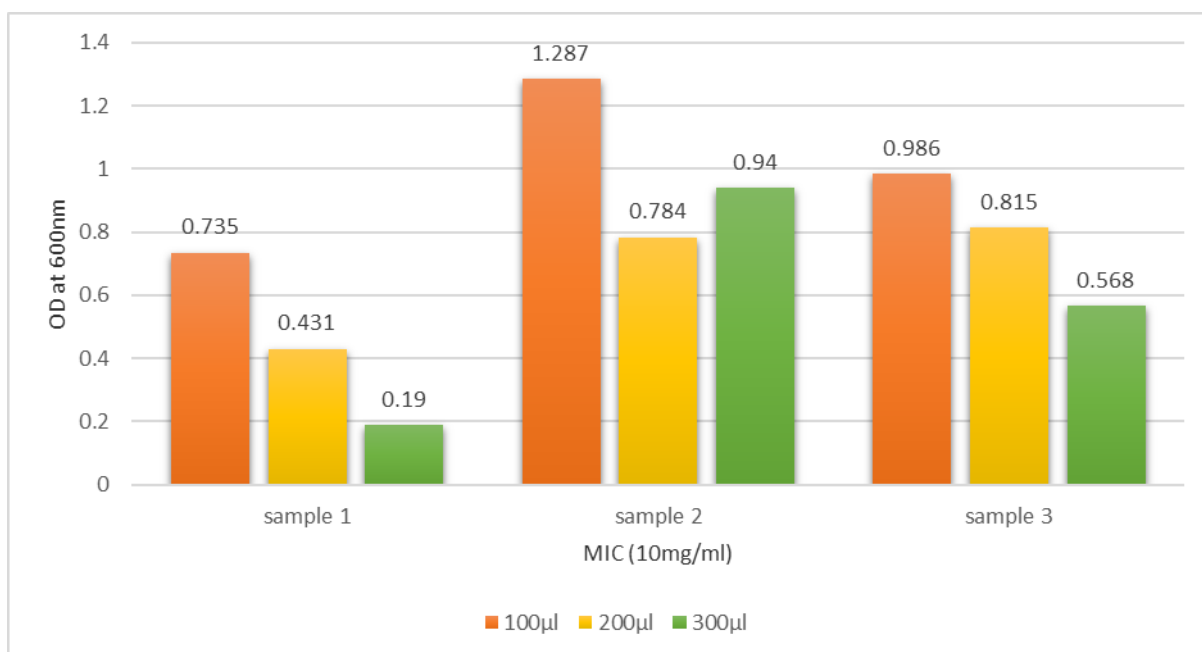


Fig 5.53 Minimum inhibitory concentration for *Proteus mirabilis*

The above graph depicts that, there is a steady decrease in the optical density

measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* showed a superior activity with OD value 0.19 against *Proteus mirabilis*.

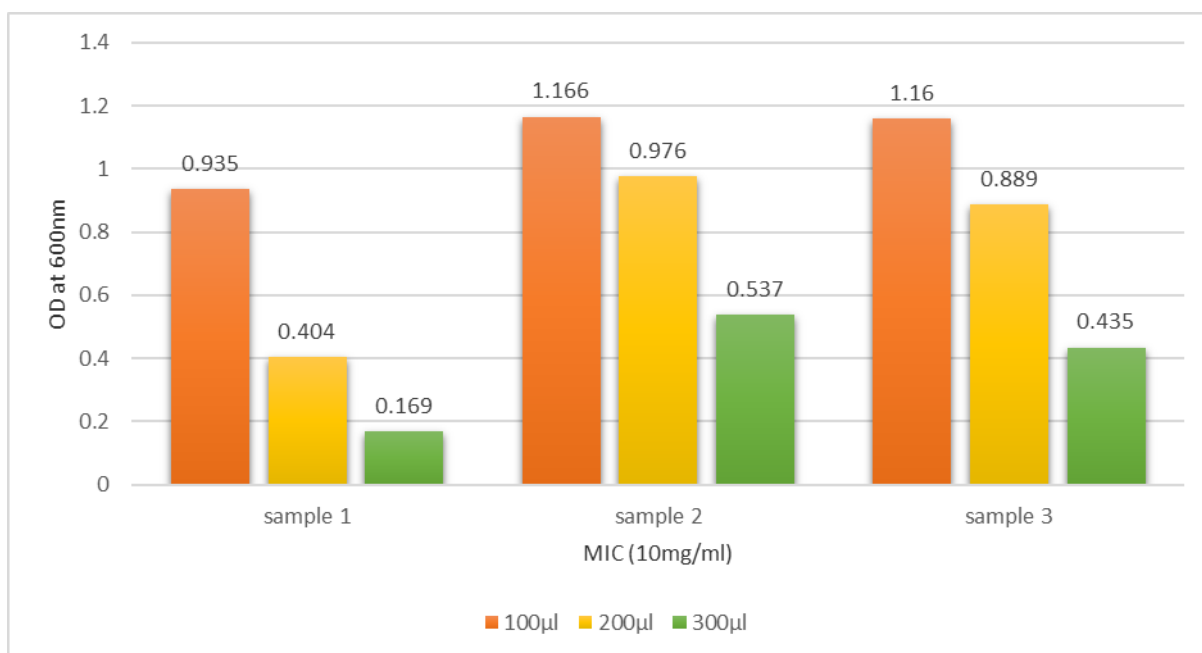


Fig 5.54 Minimum inhibitory concentration of *Proteus vulgaris*

The above graph depicts that, there is a steady decrease in the optical density measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* showed a superior activity with OD value 0.169 against *Proteus vulgaris*.

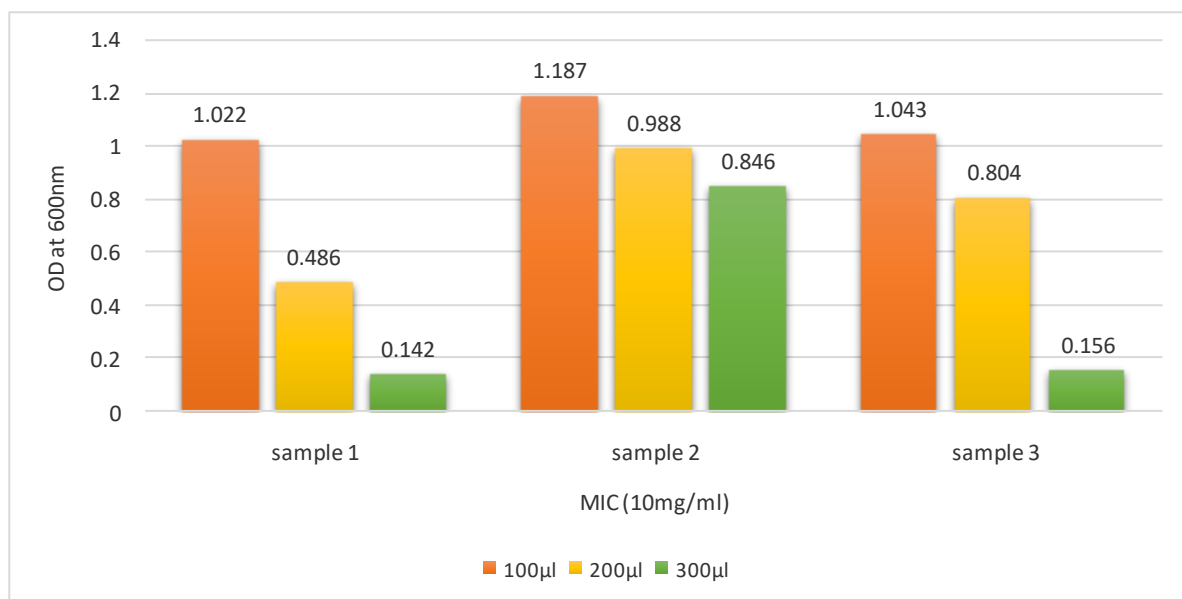


Fig 5.55 Minimum inhibitory concentration of *Pseudomonas* sp.

The above graph depicts that, there is a steady decrease in the optical density measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* and *Batis maritima* showed a superior activity at OD value 0.142 against *Pseudomonas* sp.

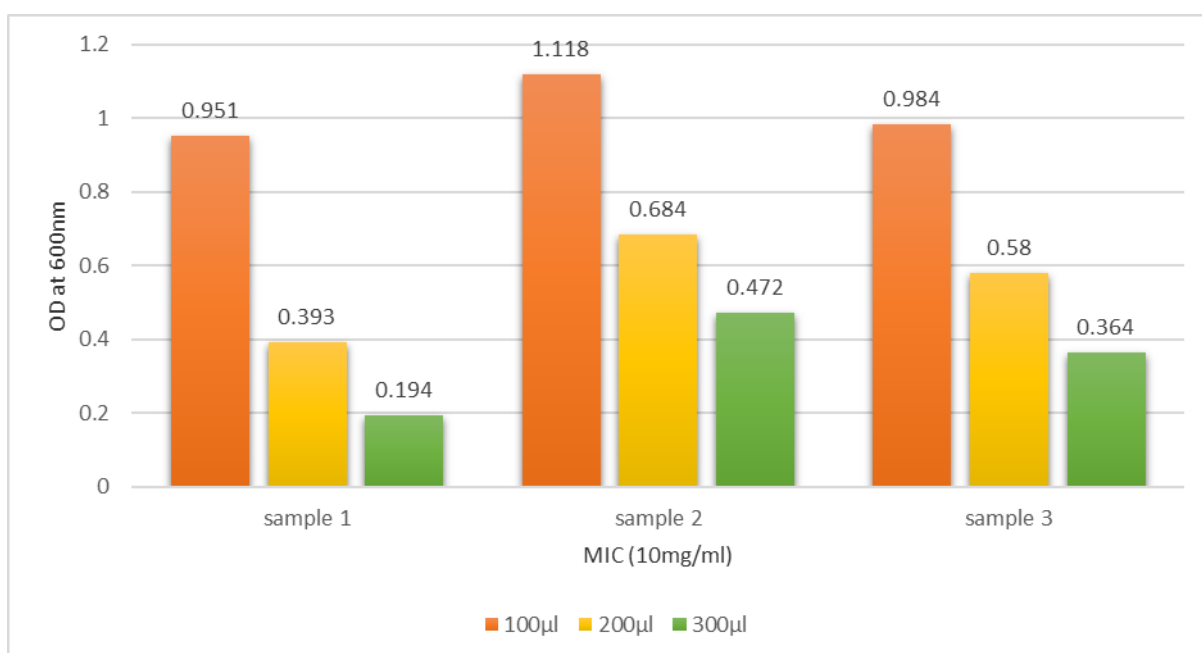


Fig 5.56 Minimum inhibitory concentration of *Staphylococcus aureus*

The above graph depicts that, there is a steady decrease in the optical density measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* showed a superior activity with OD value 0.194 against *Staphylococcus aureus*.

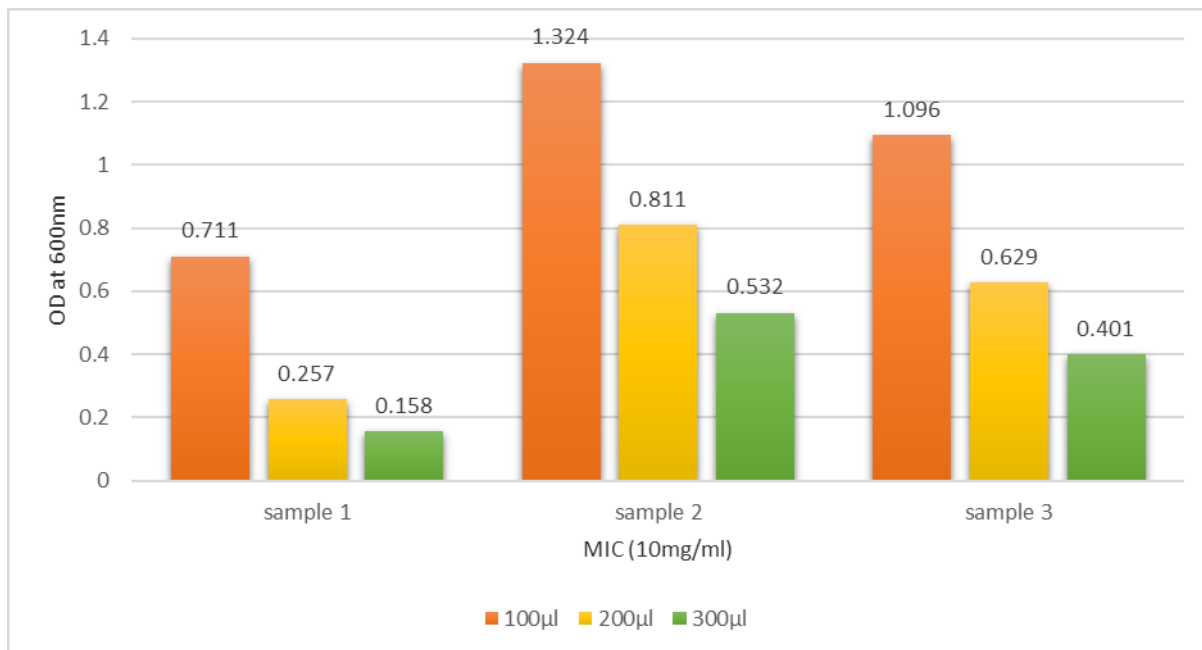


Fig 5.57 Minimum inhibitory concentration of *Vibrio sp.*

The above graph depicts that, there is a steady decrease in the optical density measured at 600nm. The methanolic extract of *Ipomoea pes-caprae* showed a superior activity with OD value 0.158 against *Vibrio sp.*

5.7 FTIR analysis:

FTIR analysis was done to determine the tentative functional group present in all three samples. The IR spectrum of samples are shown in Fig. 5.58, 5.59, 5.60 and Table 5.2. The peaks are compared with standard chart. The represented IR spectra corresponds to the presence of C-Br stretching frequency around 665.44 cm^{-1} of the functional group halo which are present in *Ipomoea pes-caprae* and *Batis maritima*. The common frequency obtained in all three samples are 1020.34 cm^{-1} , 1112.93 cm^{-1} , 1450.47 cm^{-1} and 2943.37 cm^{-1} . Frequency around 3313.71 cm^{-1} represent a secondary amine in *Batis maritima*.

Table 5.2 FTIR spectrum of samples

Name of the sample	Frequency(cm^{-1})	Bond	Functional group
<i>Ipomoea pes-caprae</i>	665.44	C-Br stretch	Halo compound
	1020.34	C-N stretch	Amine
	1112.93	C-O stretch	Secondary alcohol
	1450.47	C-H bend	Alkane
	2831.50	N-H stretch	Amine salt
	2943.37	C-H stretch	Alkane
<i>Sesuvium portulacastrum</i>	1020.34	C-N stretch	Amine
	1112.93	C-O stretch	Secondary alcohol
	1450.47	C-H bend	Alkane
	2831.50	N-H stretch	Amine salt
	2943.37	C-H stretch	Alkane
<i>Batis maritima</i>	663.51	C-Br stretch	Halo compound
	1020.34	C-N stretch	Amine
	1112.93	C-O stretch	Secondary alcohol
	1450.47	C-H bend	Alkane
	2829.57	N-H stretch	Amine salt
	2943.37	C-H stretch	Alkane
	3313.71	N-H stretch	Secondary amine

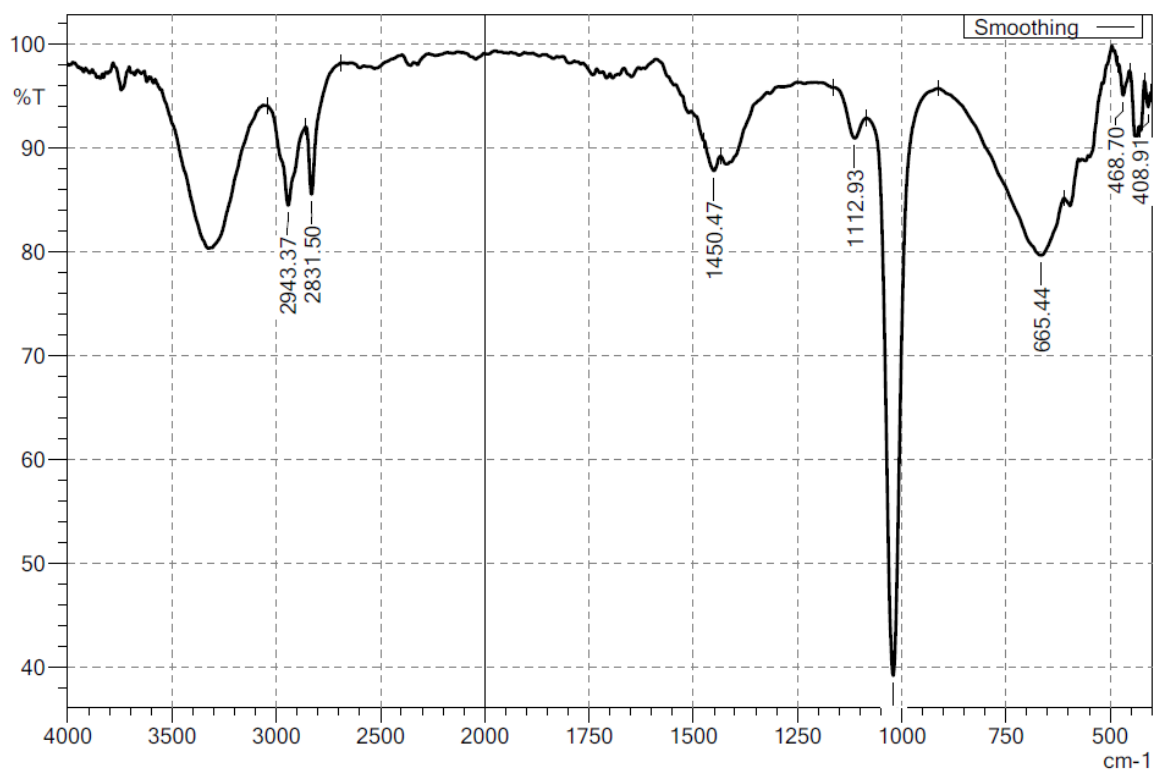


Fig 5.58 FTIR analysis of methanolic extract of *Ipomoea pes-caprae*

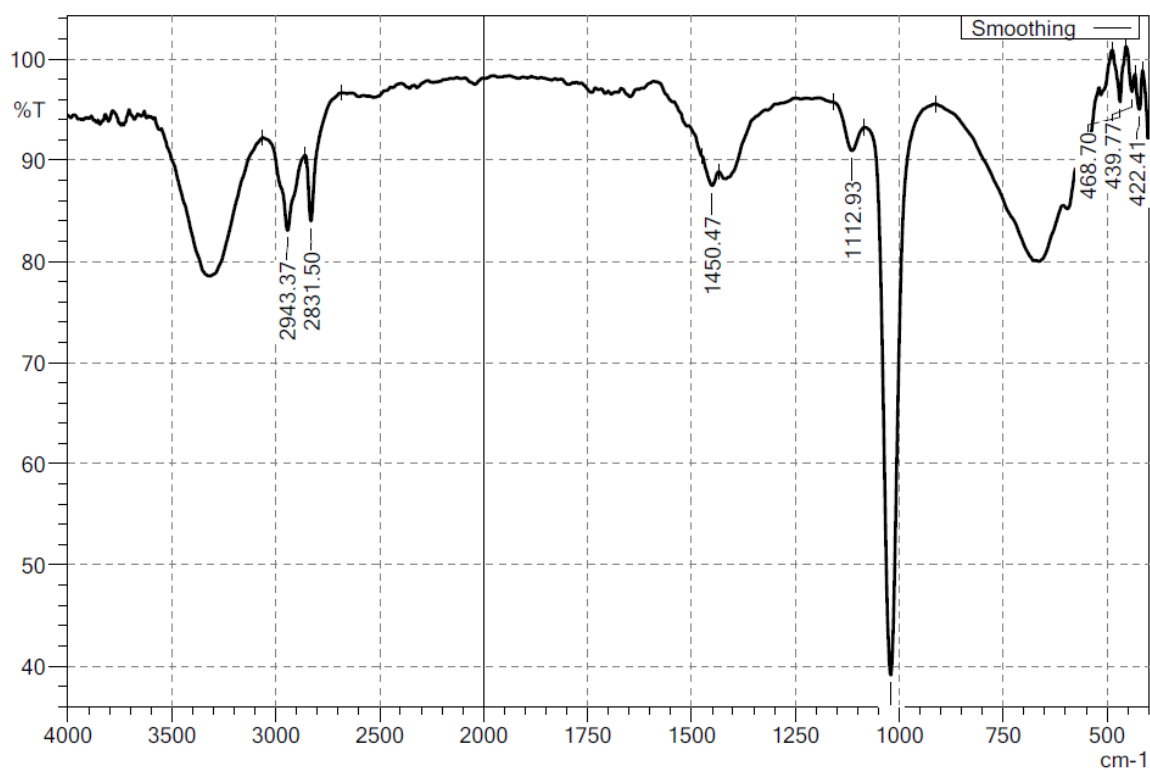


Fig 5.59 FTIR analysis of methanolic extract of *Sesuvium portulacastrum*

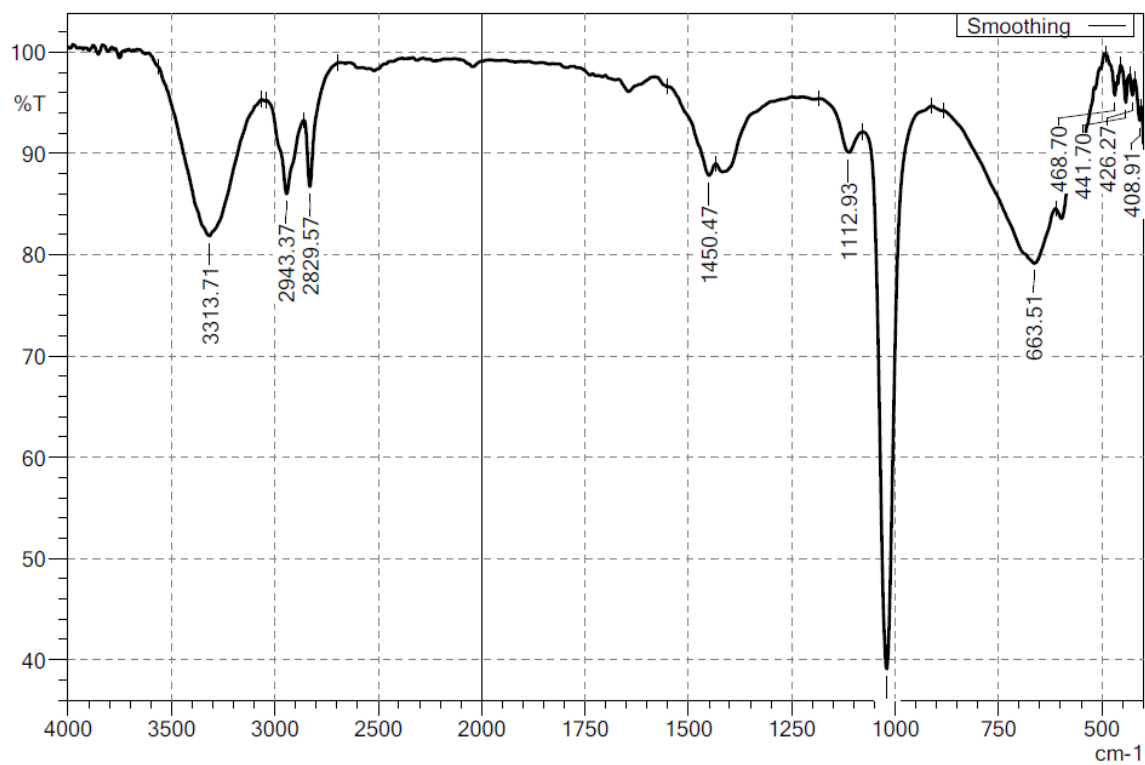


Fig 5.60 FTIR analysis of methanolic extract of *Batis maritima*

DISSCUSION:

(Sathish et al., 2016) this author has carried out his work on photochemical investigation in three salt marsh plants *Suaeda maritima*, *Salicornia brachiata* and *Sesuvium portulacastrum*. Among these plant *Suaeda maritima* has many phytochemical constituents and the methanolic extract of these plants were studied for the antibacterial activity against three human pathogenic bacteria such that *Pseudomonas aeruginosa*, *Bacillus cereus* and *E. coli* in that *Suaeda maritima* showed the maximum activity against *E. coli*, *Salicornia brachiata* shows activity against *Bacillus cereus* and *Sesuvium portulacastrum* doesn't show any activity. The extracts of these plant have high potential in pharmaceutical industry. In this present study methanolic extract of *Sesuvium portulacastrum* shows activity against *Proteus vulgaris*, *Proteus mirabilis* and *staphylococcus aureus*. Hence compared to (Sathish et al., 2016) the above study the present study shows that *Sesuvium portulacastrum* has antibacterial activity.

(Kaimudin et al., 2019) this author has done the study on three salt marsh plants namely *Ipomoea pes-caprae*, *S. alba*, *G. debilis* and *D. versluysii* among these methanolic extract of *Ipomoea pes-caprae* were shown the effective result against *E. coli*, *Klebsiella pneumoniae*, *Vibrio parahemlyticus*, and *Proteus mirabilis*. The extract was ineffective for *S. typhi*. Hence it was concluded that *Ipomoea pes-caprae* has more bioactive content and promising natural antimicrobial agents and also natural antioxidants. In the present study also *Ipomoea pes-caprae* gives maximum activity against *E. coli*, *Vibrio sp.* and *Proteus mirabilis*.

(Sen et al., 2020) this author done a work on investigating an eco-friendly, copper-free Antifouling Paint and this AP was prepared and coated on fish net which was further used for the field study analysis, and the results of the field study shown that it was just as effective in preventing biofouling on nets as the copper-based commercial antifouling paints. An experimental result also indicates that the type of raw material is an important parameter in painting processes; however, it has no role on the biofouling formation in the case of Antifouling Paint treated nets. It is important to develop biocidal agents for sustainable aquaculture that provide long-

term and non-toxic resistance to biofouling mechanisms. In the present study three substrates were taken and coated by sample mixed paint and the results of the field study shows that methanolic extract of *Ipomoea pes-caprae* mixed paint on wood gives the best result than the other substrates. Control has the high percentage of foul formation that was comparatively higher than the sample coated substrates. Hence it was concluded that this paint can be eco-friendly antifouling paint. In future it can be developed as an antifouling paint product with further analysis.

(Dahms et al., 2017) this author has done a work on isolating antifouling compounds from marine macroalgae. Red, brown, green algae were taken for the study in those green algae has less bioactive metabolites than the other two algae. Hence it was concluded that red and brown algae have high effective antifouling bioactive compound. In the present study salt marsh plants were used to investigate the antifouling activity and these salt marsh shows maximum inhibition against marine fouling organisms.

(Soliman et al., 2014) this author done a antifouling work on puffer fish (*Amblyrhynchotes hypselogenion* and *Lagocephalus sceleratus*). Crude extract was taken from ovary and mucus of the fish in different concentration which was later mixed with paint formulation and coated to the panels. These panels were kept under field trials for 24 weeks. The observed result shows that *hypselogenion* has more effect against marine fouling organisms. This was confirmed by wet weight of the panels. In the present study methanolic extract of *Ipomoea pes-caprae* (leaves) mixed with paint shows the less percentage of foul formation on wood panels compared to the control.

In the present study antifouling activity of halophytes were investigated on three plant species *Ipomoea pes-caprae*, *Sesuvium portulacastrum* and *Batis maritima*. Many researchers have been reported the antimicrobial and antioxidant activity of the salt marsh plants but this was the first work done on salt marsh plants against marine fouling organisms. Among these plants *Ipomoea pes-caprae* shows the maximum activity against *Escherichia coli*, *Proteus mirabilis*, *Proteus Vulgaris*, *Pseudomonas sp.*, *Staphylococcus aureus* and *Vibrio sp.* At the outset, the *Ipomoea pes-carpea* has shown effective antifouling activity other than two plants.

CHAPTER 6

6. SUMMARY AND CONCLUSION:

Marine biofouling is one of the major problem results in economic loss for shipping industry which is considerably huge loss in operation time, extra fuel and maintenance cost. Though heavy metal based antifouling paint prevents fouling, they are non-targeted toxic paints which cause terrible effect on marine ecosystem. Therefore, it is an essential to search for an eco-friendly antifouling paint that consists of natural resources. In this condition, the present investigation has been undertaken to find an ecofriendly antifouling compound from salt marsh plants (*Ipomoea pes-caprae*, *Sesuvium portulacastrum*, *Batis maritima*). Field study was done by mixing the three samples with commercial enamel paint separately and that was coated on the three different substrates to find percentage of fouling attachment on it. This investigation was done for 31 days and the observed result states that paint + sample I (*Ipomoea pes-caprae*) coated on wood gives the better result compared to the control (uncoated substrates). To check antifouling activity of the samples disc diffusion was performed against six marine micro biofouling organisms at different concentrations of sample (*Escherichia coli*, *Proteus mirabilis*, *Proteus Vulgaris*, *Pseudomonas sp.*, *staphylococcus aureus* and *vibrio sp.*) from this method *Ipomoea pes-caprae* shows the best result for all six organisms than the other two samples. It shows maximum activity against *Pseudomonas*. Minimum inhibitory concentration was also performed against six organisms at different concentrations. In that sample I inhibit low concentration of pseudomonas at 300µg/mL. This study states that sample I is very much effective against pseudomonas species. FTIR analysis was done to the sample. The conclusion of this study is *Ipomoea pes-caprae* (sample I) is more significant with considerable level of antifouling activity against marine biofouling organisms when compared to remaining two other salt marsh plants. These revealed results conquer that the salt marsh plants have antifouling activity obtained from naturally available coastal plants. Therefore, the salt marsh plants could be utilized as an alternative source for the production of natural antifouling paints as an eco-friendly in green environmentally by near future.

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ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION

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Technology degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY ENGINEERING SCHOOL OF BIO AND CHEMICAL ENGINEERING

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Ramya.M (37230067) who carried out the project entitled "ANTIFOULING POTENTIAL OF SALT MARSH PLANTS: AN INVESTIGATION AND APPLICATION" under our supervision from January 2021 to March 2021.

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Associate professor Associate professor

Head of the Department

Submitted for Viva voce Examination held on

Internal Examiner External Examiner

DECLARATION

I Ramya.M (37230067) hereby declare that the Project Report entitled Antifouling potential of salt marsh plants: an investigation and application done by me under the guidance of Dr. M. Masilamani Selvam, Associate Professor (Internal) and Dr. M. Arumugam, Associate Professor (External) at Center of Advanced Study in Marine Biology, Annamalai University is submitted

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ABSTRACT

Marine fouling organisms generally accumulate and deteriorate the surface of submerged ship which mainly causes low resistance and rough bottom surface on the ships. To overcome foul formation on the surface, there are so many antifouling paints available in the markets which contain highly toxic chemicals. When this toxic paint dissolves in water it may cause a harmful effect on the marine organisms and demolish the ecosystem of marine environment. So that salt marsh plants were taken and analysed for antifouling activity on marine fouling organisms. These plants are highly salt resistance. Hence this research work mainly concentrates on replacing the toxic chemicals in the paint by incorporating eco-friendly natural component into the paint which is taken from salt marsh plants.

Keywords: Fouling organisms, toxic chemical, highly salt resistance.

TABLE OF CONTENTS

CHAPTER NO. TITLE PAGE NO. ABSTRACT ABBREVIATION LIST OF FIGURES LIST OF TABLES 1 INTRODUCTION 1.1 biofilm formation 1.2 Different approaches to prevent fouling 1.3 Salt marsh 1.4 Outline of the project 2 LITERATURE SURVEY 3 AIM AND SCOPE 4 MATERIALS AND METHODS 4.1 materials 4.2 Sample collection 4.3 Solvent extraction 4.4 Field trials 4.4.1 Preparation of emulsion 4.4.2 Preparation of raw materials 4.4.3 Study area 4.5 Antifouling assays 4.5.1 Microorganisms and inoculum development 4.5.2 Antifouling activity 4.5.3 Determination of Minimum inhibitory concentration (MIC) 4.6 FTIR spectral analysis 5 RESULTS AND DISCUSSION 5.1 Sample collection 5.2 Sample preparation

5.3 Emulsion preparation 5.4 Field study results 5.4.1 After 15 days of observation 5.4.2 After 31 days of observation 5.5 Antifouling activity against marine fouling bacteria 5.6 Minimum inhibitory concentration against marine fouling bacteria 5.7 FTIR analysis 6 SUMMARY AND CONCLUSION 7 REFERENCES

CHAPTER 1

1. INTRODUCTION

Marine biofouling is caused by the adhesion of micro and macro foulers on a submerged wet surface. The environmental factors that induce the growth of fouling organisms are temperature, turbidity, waves, salinity, light, current, tides, color, pollution and texture of the substrate. The marine micro foulers are bacteria, protozoans, spores of macroalgae, diatoms and macro foulers are algae, barnacles, bryozoans, cnidarians, annelids and ascidians. A bacterium plays a major role in biofilm formation that leads to the settlement of invertebrates, sessile plants and animals on underwater structures (Nandhini. S and K. Revathi, 2016). Fouling on ship tends to hydrodynamic drag and hull roughness. Due to this reduction in speed and manoeuvrability of ship occurs (Kirschner et al., 2012).

1.1 Biofilm formation:

Marine biofilm formation generally occurs in substrates for the colonization and settlement of larvae and spores of higher sessile organisms. These microbial communities initiate the attachment of the foulers on the surface by triggering the fouling process; the interaction and specific mechanism convoluted in the initial surface adhesion are still unknown. Biofilm itself have an important role in the corrosion of surfaces immersed in seawater (Antunes, J., Leão, P., & Vasconcelos et al., 2019). Marine biofouling remains a problem with significant economic and environmental deprivation of marine transportation are increased fuel consumption up to 30% to balance the standard level of performance and this increased consumption of fuel induces the release of greenhouse gases into the environment (Garcia, Sergio, et al., 2020). Biofouling happens all over the world in various fishing equipment, offshore oil, gas industries and industries. Unguarded submerged marine metallic surfaces are mainly subjected to biofouling and corrosion (Doiron et al., 2018). The gradual fuel consumption leads to the provocation of CO₂ emission and more water transportation cost. (Abioye et al., 2019). The environmental consequences of transportation have given rise to many initiatives like greener maritime shipping and reducing the emissions from ships which affects the air quality (Davidson et al., 2018).

1.2 DIFFERENT APPROACHES TO PREVENT FOULING:

Marine fouling can be removed by three different methods namely chemical, mechanical and biological methods. Chemical methods are the most commonly used technique which includes toxic antifouling paints, foul release coating and chlorination. The toxic antifouling paints are generally used to cover the surface of the boat or ship hulls with a layer of biocides. Antifouling paints are classified into soluble and insoluble matrix paints and self-polishing copolymer paints. The Soluble matrix paints contains a soluble polymer binder with a biocide, the polymers get dispersed into water as a result the biocides are release into sea water. In insoluble matrix paints the binder used is a hard insoluble polymer that does not erode on immersion into sea. But there is chance of dispersion of biocide into water only by the dissolution of biocide particles. Self-polishing copolymer paint consists of mainly an acrylic backbone polymer as a binder with an ester linked to organometallic group. These polymers are also dissolved into water by chemical reaction (Soren Kiil, 2001). Foul-release coating contains anon-sticky property which reduces the adhesion of fouling organisms on the surface, which leads to the removal of foulds by hydrodynamic stress while navigation or just cleaning it mechanically. TBT is the most commonly used biocide. Antifouling paints incorporated with TBT (tributyl tin) was the first chemical found effective and durable against fouling organisms at 1960s. Though it is successful, due to its toxicity it harms non targeted marine organisms therefore it was banned by the government at 1970s (Christine Bressy et al., 2012). The mechanical methods are surface modification, electro-chlorination and ultrasonic system. Surface modification is method similar to the natural organism's defence against bio adhesion. Structural changes and micro topographical features can be modified on the surfaces to prevent biofouling (Bers, A. V., and Wahl, M. 2004). Electro-chlorination works on the principle of electrolysis which converts sodium chloride in the seawater into sodium hypochlorite solution to prevent biofouling (S.T. Sampathkumaran et al., 2008). Ultrasonic is device emitting mechanical waves in the ultrasonic (<20 kHz) and audible (20 Hz–20 kHz) frequency range leads to the prevention of biofouling (M. Legg et al., 2015). Some physical methods employed in biofouling are scraping method, tar and pitch (Gang Wang et al., 2020). Biological method is the addition of non-toxic or natural products from marine organisms into paint. Many marine animals are free from fouling and produce metabolites or antifouling agent which is used to prevent marine biofouling (Jgrant burgess et al., 2003).

In current scenario antifouling paints are available in the combinations of copper, zinc, tin and other heavy metals. To replace this toxic metal biogenic compounds are incorporated into the paints to produce an eco-friendly antifouling



paint.

1.3 salt marsh:

Plant cover was a primary aspect of many terrestrial systems, coastal marine and it also controls the structure of associated animal communities (Whitcraft et al., 2007). Marine plant exhibits antifungal, antibacterial, antioxidant and antifouling activities (K.Feroz khan et al., 2014). In those plants salt marshes diminishes the water waves and thus have an essential function for coastal protection. Biophysical properties of these plants play a major role in the activity of wave attenuation and may be distinguished by morphological properties such as vegetation and stem density (Schulze et al., 2019). Salt marshes are a highly valuable ecosystem which gives important to ecosystem. The global scale of marsh disappearance due to coastal squeeze and climate change, the factors affecting the edges of salt marsh by erosion are extrinsic factor such that foreshore morphology, wind exposure and the intrinsic factors are soil and vegetation (Wang et al., 2017). The occurrence of salt marsh landforms is of highly important as their connected ecosystems gives important to regulating, cultural ecosystem services and provisioning (Brooks et al., 2020). Salt marshes are widely distributed throughout the coastal area in the form of dense bunches, climbers and shrubs. The salt marsh takes up only H₂O from salt water for the growth and leave out the NaCl. It also highly tolerant to salt. The ability of salt marsh towards marine foulers needs to be tested. This study was done to assess antifouling activity from salt marsh towards marine foulers.

1.4 Outline of the project:

Sample collection

Extraction (double extraction)

Incorporation of extracts in the paint for field studies

Applying that paint on the three different substrates

Periodical checking of the painted raw materials

Checking the antifouling activity of the sample against fouling organisms.

CHAPTER 2

2. LITERATURE SURVEY:

(Nandhini. S and K. Revathi, 2016) this work was done to evaluate the antifouling potential of two mangrove species against the collected biofoulant sample. This study has shown the result that mangroves have potent AF component which can be used as a natural antifoulant.

(Kirschner et al., 2012) This review summarizes the certain effects of biofouling and explains the basis for antifouling coating design and highlights prominent advances in antifouling technologies.

(Antunes et al., 2019) this paper describes the biofilm formation and the different composition of biofilm which is isolated from the different artificial submerged surfaces via bacterial quorum sensing.

(Davidson et al., 2009) this paper compares the previous studies of how much fouling organism forms on container ship than the other submerged vessels and the risk factor to the environment by fouling organism.

(Doiron, K et al., 2018) this study was to demonstrate the function of antimicrobial peptides extracted from snow crab (SCAMPs) to reduce the formation of marine biofilms on immersed mild steel surfaces. Hence five antimicrobial peptides were found in the snow crab hydrolysate fractions were used. SCAMPs were demonstrated to interact with natural organic matter during the formation of the conditioning film and to limit the marine biofilm development in terms of viability and bacterial structure.

(García et al., 2020) this paper indicates the effective tool to study biofouling on hull properties by using four different fouling resistant coatings which were experimentally investigated to increase the hydrodynamic performance of the ship this can cause decrease in fuel consumption.

(Khan et al., 2001) this paper mainly concentrates on the health issue of ornamental fish for its sustainable growth. Therefore, five different marsh plants were taken which has high medicinal values and tested against six fish pathogens isolated from aquarium.

(Lejars et al., 2012) the work of the paper is about detailed description of the techniques and paints were used now and then for removing fouling organisms. And the concept of the work evolves in the development of ecofriendly paint.

(Schulze et al., 2019) this research work describes the biomass cultivation and efficiency of salt marsh that can attenuate the wave for the protection of coastal region.

(Wang et al., 2017) this paper talks about the factors that causing salt marsh erosion and the quantification of erodibility of sediment cores collected from the marsh edge of various salt marsh plants was done experimentally.

(Whitcraft et al., 2007) Plant cover is a fundamental feature of many coastal marine and terrestrial systems and controls the structure of associated animal communities. Both natural and human-mediated changes in plant cover influence abiotic sediment properties and thus have cascading impacts on the biotic community. Using clipping (structural) and light (shading) manipulations in two salt marsh vegetation zones (one dominated by *Spartina foliosa* and one by *Salicornia virginica*), we tested whether these plant species exert influence on abiotic environmental factors and examined the mechanisms by which these changes regulate the biotic community. In an unshaded (plant and shade removal) treatment, marsh soils exhibited harsher physical properties, a microalgal community composition shift toward increased diatom dominance, and altered macrofaunal community composition with lower species richness, a larger proportion of insect larvae, and a smaller proportion of annelids, crustaceans, and oligochaetes compared to shaded (plant removal, shade mimic) and control treatment plots. Overall, the shaded treatment plots were similar to the controls. Plant cover removal also resulted in parallel shifts in microalgal and macrofaunal isotopic signatures of the most dynamic species. This suggests that animal responses are seen mainly among microalgae grazers and may be mediated by plant modification of microalgae. Results of these experiments demonstrate how light reduction by the vascular plant canopy can control salt marsh sediment communities in an arid climate. This research facilitates understanding of sequential consequences of changing salt marsh plant cover associated with climate or sea level change, habitat degradation, marsh restoration, or plant invasion.

(Chambers et al., 2014) The control of biofouling can be achieved by a variety of methods but for an open system, such as a ship's hull, a protective paint coating is the most adopted method. The incorporation of a natural product extract directly into a coating has received little previous attention. This study has investigated a combination of the antifouling compound (a natural product extract) and the delivery system (control depletion polymer) investigated together. It was necessary to investigate the natural product incorporation into a coating and finally assess the antifouling system including the primer layers in the natural marine environment. Natural products must first be practical as antifoulant to be developed further into a functional system by their incorporation into surfaces or coatings. To demonstrate this, the natural product under investigation was homogenised into a blank proprietary antifouling paint system binder, applied to primed and unprimed ship grade steel and immersed in marine environments. Electrochemical techniques were used to investigate the effects of natural product incorporation into a coating. In addition, optical and scanning electron microscopes were used to assess the physical characteristics of the coating system. The most rigorous test for an antifouling system is a field trial. Field trials were completed at a raft exposure facility, in estuarine dock conditions at the Empress dock, National Oceanography Centre, Southampton, UK

(Dan Rittschof, 2000) this journal mainly forces on fouling of organism on the surface by molecular, microbial, and macro-organismal levels of organization. Commercially available solution to prevent foul is antifouling or foul-release coatings. Commercially available antifouling paints can effectively kill fouler, but may harm the non-targeted organisms. So, the development of natural antifoulants is preferred. The production of paints using natural product is blocked by two causes, which should be removed on the proper bases.

(Soren Kiil, 2001) this paper is about the development of self-polishing antifouling paints in a mathematical model. The model paints were prepared in different composition using copolymers and pigments. The experiment was conducted using a laboratory rotor to check the polishing rate and the amount of pigment leached by the interaction between the seawater and the artificial paint prepared.

(ChristineBressy et al., 2012) this review paper is on foul-release coating a non-toxic antifouling coating. Due to the use of biocide or toxic AF coating is used to find an alternative to this, a non-toxic AF coating was used whose efficacy depend on the low adhesion strength and a low energy is required to remove the fouling organism from the substrate. Author concludes that research is increased to produce coatings with Foul release ability particularly with the development of fluor based polymers and hybrid foul release coatings.

(A valeria bers et al., 2003) this paper is about the study of surface microtopography of various marine organisms which is less than 500 μm . scanning electron microscopy was used to characterise the surface topography of all marine organism.

Field studies were performed using a high-resolution resin of these marine natural structures to determine the influence of different microtopographies on fouling rates.

(S.T. Sampathkumaran et al., 2008) this paper is based on the use of electro chlorination system a method to prevent biofouling in seawater. Biofouling in pipe lets can be prevented using chemical treatment by converting sodium chloride to sodium hypochlorite. Chlorine is the frequently used disinfectant to prevent fouling.

(M. Legg et al., 2015) this review is about the use of acoustic techniques to inhibit biofouling, mainly using a low ultrasonic frequency. Many laboratory and sea trial studies have reported a successful prevention of biofouling.

(Gang Wang et al., 2020) their work is on the cleaning of fouling organism from the marine substratum by using scraping method. This method is proposed on the principle of orthogonal cutting theory and the peel zone method. Removing of barnacles depend on the blade rake angle. Therefore, a reduction in the cutting force can be achieved by increasing the blade rake angle.

(Jgrant burgess et al., 2003) their research is to produce a non-toxic antifouling paint. They have extracted some metabolites from marine epiphytic bacteria isolated from marine environment. These metabolites were incorporated into paint resin and exposed to field studies.

(Dahms H. U and Hellio C, 2009) this literature is on the laboratory bioassays for the screening of activity and toxicity of potential antifouling compounds. Various methods are explained in this paper from bioassay for microbial fouling to macro fouling.

(Sivan Nir, 2016) their review is on the development of non-toxic antifouling approaches. Their main focus is on the latest advances in the field of antifouling materials. The advanced techniques which are discussed here are Surface topography to mimic the surface topography of marine organisms or plants, DOPA-based antifouling materials an adhesive protein of marine mussels can be used as bio adhesive coating and Quorum-sensing inhibitory compounds as antifouling materials to inhibit the bacterial communication.

(Aseer Manilal et al., 2010) their research was on the antifouling potentials of seaweed against marine fouling organisms. The red sea algae showed a broad range of activity, which was further, purified by column chromatography and identified for specific compound using GC-MS.

CHAPTER 3

3. AIM AND SCOPE:

- To prepare an emulsion containing methanolic extract and commercial enamel paint.
- Field study for the painted substrates at estuary.
- To evaluate the antifouling activity of extracted sample against the marine fouling organisms.
- To study the functional group of the methanolic crude extract using by FTIR.
- To determine the minimum inhibitory concentration of methanolic crude extract.

CHAPTER 4

4. MATERIALS AND METHODS:

Methanolic extracts of three salt marsh plants were tested against marine fouling bacteria. These extracts were also incorporated into the paints for field trials.

4.1 Materials:

1. solvent: Methanol

2. Plant sample:

- Sample 1 – Ipomoea pes-carpea
- Sample 2 – Sesuvium portulacastrum
- Sample 3 – Batis maritima

3. Ware: Mortar and pestle, Conical flask, Funnel, Measuring cylinder, Petri plates, Whatman filter paper no: 1, Aluminum foil paper, Micropipettes, Pipette tips, Cotton swab, Test tubes, Test tube stand, Bunsen burner, Nylon rope, Asian enamel paint 500ml (blue), Thinner 200ml, Paint brush.

4. Instruments: Shaker (Remi Limited), Rotary evaporator (Cyber lab), Laminar air flow chamber, lyophilizer (), FTIR (Shimadzu), Autoclave, UV spectroscopy (Molecular Devices).

5. Substrates

- Wood
- Aluminium sheet
- Fibre sheet

6. Media:

1. Mueller Hinton agar:

- Mueller Hinton agar - 32 g/l
- Agar - 10 g/l

2. Mueller Hinton broth:

- Mueller Hinton broth - 38g/l

3. Nutrient broth:

- Nutrient broth - 13g/l

4. Zobell agar

- Zobell agar - 55.25g/l
- Agar - 10g/l

4.2 Sample collection:

Three salt marsh plants were collected from southeast coast of India. Ipomoea pes-carpea and sesuvium portulacastrum from the coastline of singarathoppu(). Batis maritima from the coast area of parangipettai.

4.3 Solvent Extraction:

Solvent extraction was carried out as previously reported (K.Feroz khan et al., 2014) with appropriate modification. The plant leaves were washed with sea water subsequently with tap water to remove the dirt. The washed leaves were pat dried to remove the moisture content. 100g of fresh leaves were weighed and crushed using mortar and pestle. The crushed samples were soaked in 400ml of methanol (1: 4 ratios) and kept in the shaker(300c) for 36 hours at 100 rpm. The

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plant extracts were filled through Whatman No.1 filter papers. The extracts were condensed

by using rotary evaporator and lyophilizer. The obtained condensed extracts were stored for further assay.

4.4 field trials:

4.4.1 Preparation of Emulsion:

Enamel paint were chosen for the study, because it is commonly used paint to coat the submerged structures. In this study, Asian enamel paint was incorporated with different types of solvents and samples at the ratio of 4:1(40ml+10ml). Five separate beakers were taken to check the solubility of the paint namely,

Beaker A - paint + thinner

Beaker B - paint + solvent(methanol)

Beaker C - paint + Ipomoea pes-carpea extract

Beaker D - paint + Sesuvium portulacastrum extract

Beaker E - paint + Bastis maritima extract

4.4.2 Preparation of raw materials:

The raw materials were used are Wood, Aluminium and Fibre sheets. These materials were cut in the dimension of 6*6 inches. The prepared paint was double coated on the raw materials using paint brush. The paint coated raw materials were shade dried for 10 days.

4.4.3 Study area:

The dried substrates were tied using a nylon rope at vellar estuary, opposite to marine biology station, parangipettai (11029'N,79046'E). substrates were partially submerged at lowest low tide region. The tidal range in this zone is 1m with the average depth of 2.5m. Nature of the substratum is sandy-muddy which comprises less than 20% slit and higher clay content with the salinity range of 28.13 ppt (ENVIS).

4.5 Antifouling assays:

4.5.1 Microorganisms and Inoculum development:

The crude methanolic extracts were test against the bacterial pathogens namely *Pseudomonas* sp., *Vibrio* sp., *Proteus vulgaris*, *Proteus mirabilis*, *E. coli* and *staphylococcus aureus*. All microorganisms were collected from medical college, Annamalai university, Chidambaram.

The bacterial strains were sub cultured in 5ml of nutrient broth. The strains were inoculated in marine zobell agar to observe the single bacterial colonies after 24 h.

4.5.2 Antifouling activity:

Antimicrofouling activity using methanolic extract was performed as previously described (S. Satheesh et al., 2012) with right changes. Antibacterial activity was carried out using disc diffusion method against six biofouling bacteria. Mueller Hinton agar was prepared, sterilized and 20ml of media was poured to each sterile Petri plates aseptically. After solidification, 10µl of bacterial culture was swabbed using a cotton swab onto the agar medium and allowed to dry for 5 minutes. The disc was made with Whatman No: 1 filter paper with 6mm in diameter. These discs were impregnated in the Petri plates. Different concentration of crude extracts of 100µl,150µl and 200µl were loaded onto the disc. Tetracycline

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was used as a control. The plates were incubated at 37°C for 24 h. After incubation, zone of inhibition was observed

around the disc and the zone size was measured in millimeters.

4.5.3 Determination of Minimum inhibitory concentration (MIC):

Minimum inhibitory concentration was carried as described (Fabio S. Murakami et al., 2019). Minimum inhibitory concentration (MIC) was used to determine the lowest concentration of the sample required to inhibit the growth of the bacterial strains. MIC for different bacterial strains were performed using different concentrations. The sterile test tubes were filled with 5ml of Mueller Hinton broth and different concentration of crude extracts (100 µg/ml, 200 µg/ml and 300µg/ml) was added. These test tubes were incubated with 10µl of bacterial inoculum from 24 h old culture. The turbidity was observed after 24 h using UV-spectroscopy at 600nm and the results were tabulated.

4.6 FTIR spectral analysis:

The identification of functional groups of the sample was obtained by using Fourier transform infrared spectroscopy. The study uses an infrared radiation on the sample which causes an atomic vibration of a molecule present in the sample,

using known concentration of the extract. A small drop of sample was placed on KBr plate and using second plate a thin even film was made. Plates were placed into the sample holder and run a spectrum.

CHAPTER 5

5.RESULT AND DISSCUSION:

5.1 sample collection

Fig 5.1 Ipomoea pes-carpea

Fig 5.2 Sesuvium portulacastrum

Fig 5.3 Batis maritima

5.2 sample preparation

Fig 5.4 Samples were crushed by using motor and pestle method

Fig 5.5 crushed samples kept in Shaker for incubation

Fig 5.6 samples after incubation

5.3 Emulsion preparation:

Fig 5.7 Asian enamel paint and Thinner

Fig 5.8 Paint + Thinner Fig 5.9 Paint + Solvent

Fig 5.10 Paint + Sample 1 Fig 5.11 Paint + sample 2

Fig 5.12 Paint + sample

Fig 5.13 Raw materials

Fig 5.14 Controls (uncoated substrates)

Fig 5.15 Paint and thinner coated substrates

Fig 5.16 Paint and solvent coated substrates

Fig 5.17 Paint and sample 1 coated substrates

Fig 5.18 paint and sample 2 coated substrates

Fig 5.19 Paint and sample 3 coated substrates

Fig 5.20 the full picture of substrates before immersing at estuary

Fig 5.21 the tied substrates immersed at lowest low tide of the estuary

Fig 5.22 shows location site where the field study was performed

5.4 Field study results:

5.4.1 After 15 days of observation:

(A) (B) (C)

Fig 5.23 indicates uncoated substrates kept for control (A) wood, (B) Aluminium sheet, (C) Fibre sheet.

(A1) (B1) (C1)

Fig 5.24 shows the sample 1 and paint coated substrates (A1) sample 1 + paint coated wood, (B1) sample 1+ paint coated aluminium sheet and (C1) sample 1 + paint coated Fibre sheet.

(A2) (B2) (C2)

(A3) (B3) (C3)

Fig 5.26 shows sample 3 + paint coated substrates (A3) sample 3+ paint coated wood, (B3) sample 3+ paint coated aluminium sheet and (C3) 3 + paint coated fibre sheet.

(A4) (B4) (C4)

Figs 5.27 shows Thinner + paint coated substrates (A4) thinner + paint coated wood, (B4) thinner + paint coated aluminium sheet and (C4) thinner + paint coated fibre sheet

(A5) (B5) (C5)

Fig 5.28 shows Solvent + paint coated substrates (A5) Solvent + paint coated wood, (B4) Solvent + paint coated aluminium sheet and (C4) Solvent + paint coated fibre sheet.

5.4.2 After observation of 31 days:

(A6) (B6) (C6)

Fig 5.29 indicates the uncoated control (A6) wood, (B6) aluminium sheet and (C6) fibre sheet

(A7) (B7) (C7)

Fig 5.30 shows sample 1 + paint coated substrates, (A7) sample 1 + paint coated wood, (B7) sample 1 + paint coated aluminium sheet and (C7) sample 1 + paint coated fibre sheet.

(A8) (B8) (C8)

Fig 5.31 shows sample 2 + paint coated substrates, (A8) sample 1 + paint coated wood, (B8) sample 2 + paint coated aluminium sheet and (C8) sample 2 + paint coated fibre sheet.

(A9) (B9) (C9)

Fig 5.32 shows sample 3 + paint coated substrates, (A9) sample 3+ paint coated wood, (B9) sample 2 + paint coated aluminium sheet and (C9) sample 3 + paint coated fibre sheet .

Fig 5.33 shows solvent +paint coated substrates wood, aluminium sheet and fibre sheet.

(A9) (B9) (C9)

Fig 5.34 shows thinner + paint coated substrates, (A9) thinner + paint coated wood, (B9) thinner + paint coated aluminium sheet and (C9) thinner + paint coated fibre sheet.

From the field study fig 5.21 shows better result for paint + sample 1 coated wood when compared to control the uncoated substrates fig 5.28 shows fouling formation. Hence sample 1 shows better activity for 31 days of observation. Therefore, salt marsh plant has activity against marine fouling organisms. Sample 3 and sample 2 shows moderate activity.

5.5 Antifouling activity against marine fouling organism at various concentrations by disc diffusion methods:

Antifouling assay has done against six organisms using three samples at 100, 150 and 200 micro litre concentrations. In that sample 1 forms zone of inhibition up to 26 mm at 200 micro litre concentration and the control used here is antibiotic tetracycline to compare the activity of the sample

Fig 5.35 shows the plate without culture swab (control)

(A10) (B10) (C10)

Fig 5.36 shows zone of inhibition formed at various concentrations against *Porteus vulgaris*, (A10) sample 1, (B10) sample2 and (C10) sample 3

(A11) (B11) (C11)

Fig 5.37 shows zone of inhibition formed at various concentrations against *Staphylococcus aureus*, (A11) sample 1, (B11) sample 2 and (C11) sample 3

Fig 5.38 shows zone of inhibition formed at various concentrations against *Escherichia coli*, (A12) sample 1, (B12) sample 2 and (C12) sample 3.

(A13) (B13) (C13)

Fig 5.39 shows zone of inhibition formed at various concentrations against *Porteus mirabilis* (A13) sample 1, (B13) sample 2 and (C13) sample 3

Table 5.1 indicates zone of inhibition formed at various concentrations against six Marine fouling organisms and Tetracycline (Antibiotic) was given as control to compare the activity of the sample

species sample 1 Sample 2 Sample 3

Escherichia coli 100 150 200 C 100 150 200 C 100 150 200 C 18

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mm 20 mm 24 mm 14 mm - - - 14 mm 11 mm 12 mm 14 mm 11 Mm <i>Proteus mirabilis</i> 13 mm 15 mm 19 mm 12 mm 9 mm 10 mm 12 mm 11 mm - - - 13 mm <i>Proteus vulgaris</i> 13 mm 24 mm 25 mm 14 mm 10 mm 12 mm 13 mm 12 mm 10 mm 12 mm 14 mm 12 mm <i>Pseudomonas</i> 21 mm 22 mm 26 mm 16 mm - - - 9 mm 18 mm 21 mm 23 mm 15 Mm <i>Staphylococcus aureus</i> 9 mm 11 mm 13 mm 10 mm - - - 13 mm 16 mm - 9 mm 13 mm 11 Mm <i>Vibrio</i> 20 mm 19 mm 23 mm 9 mm - - - 13 mm 8 mm 10 mm 11 mm 13		

mm

(

A14) (B14) (C14)

Fig 5.40 shows zone of inhibition formed at various concentrations against *Vibrio* (A14) sample 1, (B14) sample 2 and (C14) sample 3

(A15) (B15) (C15)

Fig 5.41 shows zone of inhibition formed at various concentration against *Pseudomonas* (A15) sample 1, (B15) sample 2 and (C15) sample 3

5.6 Minimum Inhibitory Concentration against marine fouling bacteria at various concentrations

(A16) (B16) (C16)

Fig 5.42 turbidity level at different concentration against *Proteus Vulgaris* (A16) sample 1, (B16) sample 2, (C16) sample 3.

(A17) (B17) (C17)

Fig 5.43 turbidity level at different concentration against *Staphylococcus aureus* (A17) sample 1, (B17) sample 2, (C17) sample 3.

(A18) (B18) (C18)

Fig 5.44 turbidity level at different concentration against *Escherichia coli* (A18) sample 1, (B18) sample 2, (C18) sample 3.

(A19) (B19) (C19)

Fig 5.45 turbidity level at different concentration against *Proteus mirabilis* (A19) sample 1, (B19) sample 2, (C19) sample 3.

(A20) (B20) (C20)

Fig 5.46 turbidity level at different concentration against *Vibrio* sp. (A20) sample 1, (B20) sample 2, (C20) sample 3.

(A21) (B21) (C21)

Fig 5.47 turbidity level at different concentration against *Pseudomonas* sp. (A21) sample 1, (B21) sample 2, (C21) sample 3.

Fig 5.48 graph represents the inhibitory concentrations of *Escherichia coli*

Fig 5.49 graph represents minimum inhibitory concentration for *Proteus mirabilis*

Fig 5.50 graph represents the minimum inhibitory concentration of *Proteus vulgaris*

Fig 5.51 graph represents the minimum inhibitory concentration of *Pseudomonas*

Fig 5.52 graph shows minimum inhibitory concentration of *Staphylococcus aureus*

Fig 5.53 graph represents the minimum inhibitory concentration of *Vibrio*

CHAPTER 6

6.SUMMARY AND CONCLUSION:

Marine biofouling is one of the major problem results in economic loss for shipping industry which is considerably huge loss in operation time, extra fuel and maintenance cost. Though heavy metal based antifouling paint prevents fouling, they are non targeted toxic paints which cause terrible effect on marine ecosystem. Therefore, it is an essential to search for an eco friendly antifouling paint that consists of natural resources. In this condition, the present investigation has been undertaken to find an ecofriendly antifouling compounds from salt marsh plants (*Ipomoea pes-carpea*, *Sesuvium portulacastrum*, *Batis maritima*). Field study was done by mixing the three samples with commercial enamel paint separately and that was coated on the three different substrates to find percentage of fouling attachment on it. This investigation was done for 31 days and the observed result states that paint + sample 1 (*Ipomoea pes-carpea*) coated on wood gives the better result compared to the control (uncoated substrates). To check antifouling activity of the samples disc diffusion was performed against six marine micro biofouling organisms at different concentrations of sample (*Escherichia coli*, *Proteus mirabilis*, *Proteus Vulgaris*, *Pseudomonas*, *staphylococcus aureus* and *vibrio*) from this method *Ipomoea pes-carpea* shows the best result against all six organisms than the other two samples. It shows maximum activity against *Pseudomonas*. Minimum inhibitory concentration was also performed against six organisms at different concentrations. In sample 1, the lowest concentration required to inhibit the growth of *Pseudomonas* sp. is 300µl(10mg/ml). This study states that sample 1 is very much effective against *pseudomonas* sp. compared to other samples. The IR spectra of methanolic extract of different salt marsh plants showed various frequency around the range 400-3500 cm⁻¹. Sample (*Ipomepea pes-carpea* and *batis maritima*) represents the halo compound around the region 665.44 cm⁻¹. The conclusion of this study is *Ipomoea pes-carpea* (sample1) is very much effective and shows some antifouling activity against marine biofouling organisms than the other two salt marsh plants. This states that salt marsh plants may have antifouling activity which is naturally available in coastal area. Future work is to separate the antifouling compound present in the salt marsh plants. Therefore, in future by using salt marsh plants an eco-friendly antifouling paint product can be developed.

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Chart1

sample 1 sample 1 sample 1 sample 2 sample 2 sample 2 sample 3 sample 3 sample 3 0.711 0.257 0.158 1.324 0.811 0.532 1.096 0.629 0.401

Sheet1

100µg/ml 200µg/ml 300µg/ml sample 1 0.711 0.257 0.158 sample 2 1.324 0.811 0.532 sample 3 1.096 0.629 0.401

100µg/ml 200µg/ml 300µg/ml MIC(µg/ml) OD at 540nm *Vibrio* sp

Chart1

sample 1 sample 1 sample 1 sample 2 sample 2 sample 2 sample 3 sample 3 sample 3 0.951 0.393 0.194 1.118 0.684 0.472 0.984 0.58 0.364

Sheet1

100µg/ml 200µg/ml 300µg/ml sample 1 0.951 0.393 0.194 sample 2 1.118 0.684 0.472 sample 3 0.984 0.58 0.364

100µg/ml 200µg/ml 300µg/ml MIC(µg/ml) OD at 540nm *S.aureus*

Chart1

sample 1 sample 1 sample 1 sample2 sample2 sample2 sample 3 sample 3 sample 3 1.022 0.486 0.142 1.187 0.988 0.846 1.043 0.804 0.532

Sheet1

100µg/ml 200µg/ml 300µg/ml sample 1 1.022 0.486 0.142 sample2 1.187 0.988 0.846 sample 3 1.043 0.804 0.532

100µg/ml 200µg/ml 300µg/ml MIC (µg/ml) OD at 540nm *Pseudomonas* sp

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plant extracts were filled through Whatman No.1 filter papers. The extracts were condensed		plant extracts from different solvents were filtered through Whatman No. 1 filter papers. The extracts were condensed		
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	was used as a control. The plates were incubated at 37°C for 24 h. After incubation, zone of inhibition was observed		was used as a blind control in these The plates were incubated at 37°C for 24 urs. zone of inhibition was observed	
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	Burgess, J. G., Boyd, K. G., Armstrong, E., Jiang, Z., Yan, L., Berggren, M., and Adams, D. R. (2003). The development of a marine natural product-based antifouling paint. Biofouling, 19(			
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10/13	SUBMITTED TEXT	32 WORDS	100% MATCHING TEXT	32 WORDS
	Davidson, I. C., Brown, C. W., Sytsma, M. D., and Ruiz, G. M. (2009). The role of containerships as transfer mechanisms of marine biofouling species. Biofouling, 25(7), 645-655 9.			
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8/13	SUBMITTED TEXT	19 WORDS	76% MATCHING TEXT	19 WORDS
	G., Ramamoorthy, K., and Sugesh, S. (2013). Antibacterial activities of salt marsh plants against marine ornamental fish pathogens.		G. Sankar, K. Ramamoorthy and S. Sugesh, 2013. Antibacterial Activities of Salt Marsh Plants Against Marine Ornamental Fish Pathogens.	
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