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EVALUATION OF TREE BARKS FOR ANTI- FOULING PROPERTIES – A PILOT STUDY

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Abstract

Fouling has been a major complication that has caused many hazardous effects on the environment and also to mankind .Earlier, prevention of fouling was done using antifoulant chemicals like tributylin (TBT), triphenyltin cuprous oxide etc, which found to cause certain effects like damaging non-target organisms of commercial value in coastal values. Hence, measures were taken to reduce any such damage by ingenious means of producing antifoulants that do not harm the ecosystem. This article discusses the methods of preventing biofilm formation by using environmental friendly natural ingredients among which the bark of *Moringaolifera* was chosen to test their ability for inhibiting biofilm formation.

Keywords: fouling, antifoulants , biofilm, barks.

INTRODUCTION

Adhesion of marine fouling organisms on artificial surfaces such as ship hulls causes many problems, including extra energy consumption, high maintenance and increased corrosion. Therefore marine antifouling play a major role in marine environment. Marine fouling should be controlled to increase the efficiency of the ship and reduce the energy consumption. Antifouling paints have the ability of control the fouling mechanism in the ship hulls. Fouling happens in two stages such as Micro-fouling and Macro-fouling.

Fouling could be prevented or removed by a process called Antifouling in which non-toxic mechanical strategies are used in the prevention of organisms from attaching to the bottom of the ship hulls. One such method is using antifoulants as paints such

as TBT (Tributylin) that acts against barrier. But due its toxic effect in the surrounding environment IMO (International Maritime Organisation) has banned its usage. The most commonly used antifouling pigment-cuprous oxide, also causes certain harmful effects, hence alternatives are being invented.

Biofilms are slimy substance that is caused due to the adhesion of bacterial cells that modify the surface physiochemical properties, influencing the adhesion of successive colonizers such as algae cyanobacteria and protists (Wetzel, 2001; Dang and Lovell, 2016). In fact, bacteria is found to be the major microbe on marine surfaces to determine the structure and function of the mature biofilm (Dang et al., 2008; Dang and Lvell., 2016). When a biofilm is formed, membrane cleaning may represent 5-20% of operating costs and may be performed by chemical cleaning agents such as acids, metal chelating agents and enzymes (Maddah and Chogle, 2017). This is another major cause for fouling to occur.

MARINE FOULING

The work was to examine the antimicrobial properties of soft coral *Sinularia* sp. against marine biofilm-forming bacteria and the result is bioactive substances produced by soft corals and is great possibility to find alternatives for metal based coating. Much research has been undertaken in order to find the secondary metabolites which may be used as antifoulant (Radjasa et al., 2001). Foul release coatings on the underwater hulls and propellers of ships are a new way to improve efficiency. They have proven to provide better fouling control than SPC antifouling. These result in increased fuel efficiency, reduced engine spare parts and reduced maintenance costs (Bader et al., 2002). In considering the prevention of the new catastrophe in the future and the conservation of the world marine environment for a long time and ban may not provide a simple solution (Mizuno et al., 2002). The risk posed by biofouling comes not only from the introduction and establishment of adult organisms in the new location, as a result of, for example, dislodgement from the vector, but also as a consequence of the fact that most biofouling species have planktonic life stages and that spawning can be induced by the altered environmental conditions in a port or marina (Minchin and Gollasch, 2003). The anti-fouling systems which have been developed have largely been based on the use of biocides, which has implications for the broader marine environment, and which concerns have led to the imminent ban of the most effective of those biocides, namely TBT (Jackson et al., 2008). The enzymes claimed to hold antifouling activity are classified according to catalytic functions. The enzyme functions are juxtaposed with the current knowledge about the chemistry of settlement and adhesion of fouling organisms. Specific focus will be on

bacteria, microalgae, invertebrate larvae and macroalgae zoospores. The ultimate test of the antifouling potential of various enzymes will be whether they can successfully be incorporated in feasible, effective and durable marine coatings (Jakob et al., 2008). Most anti-fouling coatings in use are based on leakage of toxic substances. The intention of this leakage is to prevent fouling organisms. However, also non-target organisms may be affected. We have studied the effect of leakage water from several anti-fouling coatings for use on pleasure boats or for ships. Some coatings used copper as active agent whereas other coatings were so called biocide-free, functioning by physical means. A test battery has been used with organisms representing the bacteria (*Vibrio fisheri*), algae (*Ceramiumten uicorne*) and crustaceans (*Nitocra spinpes*) (Gothenburg et al., 2009). The current status of the research into the role of EPS in bacterial attachment followed by biofilm formation. The latter has a profound impact on an array of biomedical, biotechnology and industrial fields including pharmaceutical and surgical applications, food engineering, bioremediation and bio-hydrometallurgy. The expansion of knowledge in relation to molecular mechanisms involved in bacterial-mineral attachment may be relevant in the enhancement of bioleaching timing and efficiency (Barbara et al., 2009).

BIOFILM FORMATION

The *Pseudoalteromonas* 3J6 culture supernatant is able to impair biofilm formation of most of the marine bacteria tested. Further experiments are carrying out to purify and identify the anti-biofilm molecules secreted by *Pseudoalteromonas* 3J6 (Klein et al., 2009). Hull roughness due either to the presence of a coating or to hull fouling incurs an operational cost to the vessel due to increases in shaft power to reach a given speed and associated increases in fuel consumption (Schultz et al., 2010). Biofouling protection for marine in situ sensors is a complex problem. Since the quality of the measurement is involved, the fouling protection, especially on the sensitive part of the sensor, must be very effective (Delauney et al., 2010).The most promising approaches include technical hygiene (“good housekeeping”) to minimize the fouling potential of the water phase, for example, by keeping bacterial numbers as well as nutrients as low as possible (Flemming et al., 2011). The present study represents the potential of bacterial symbiont of sea grass species *Thalassia hemprichii* and *Enhalus acoroides* as the alternative source of environmentally friendly marine antifoulant. The so far un-characterized secondary metabolites from these active bacterial symbionts deserve futher work on the bioassay- guided isolation and purification of the active antifouling compounds (Marhaeni et al.,

2011). Biofouling represents a complex mechanism where the quality of the feed water, the physico-chemical properties of the membrane and the operating conditions all play a role. Biofouling begins with the attachment of microorganisms to the membrane surface leading to the formation of a bio film layer (Thang et al., 2012). The Metallic antifouling coatings are of serious environmental concern. Since the banning of TBT based AF coatings, copper is widely used in AF formulations. However, there is growing evidence that copper released from these coatings is highly detrimental to the marine environment (Madhu et al., 2012). The bio film of *Psuedomonas fluorescens* FF48 was effective in controlling the formation of *Flavobacterium psychrophilum* bio films in vitro. The bio film is the state in which microorganisms are highly persistent in the environment; therefore when a bio controller is to be applied, the interaction between the antagonist-pathogen bio films must be considered. In this work, the ideal situation is to ensure the prior establishment of the bio controller in the cultivation system before it is confronted with the pathogen, to prevent the latter from adhering and persisting in the environment (Mery et al., 2013). Fabricated self-lubricating biofouling-release surfaces designed to mimic natural self-replenishing materials (Caitlin et al., 2014).The most research during the last few decades is focusing on physical and chemical defences of natural non-fouling surface. Many naturally derived compounds are used like enzymes, degradable and repellent compounds for control the bio film formation (Todorkavladkova et al., 2014). The surface patterning ,enzyme alternatives of toxic biocides and nature derived anti- bio film agents(natural biocides, bio surfactants/ dispersants and quorum sensing inhibitors) are the used in the bio film reduction process(Todorkavladkova et al., 2014). The work provides the effect for four “tin free” SPC antifouling paints on *Artemia* nauplii development as a result the estimation of the toxicity of antifouling paints tested to the product as a whole is toxicologically important and other agents for controlling leaching rates include the formation can influence anti-foulants toxicity as they have synergistic effects (Jane et al., 2014). The objective of this research paper is to prevent and inhibit the formation of bio films, managing bio films, advances in bio film research for quality drinking water, public health implications of bio films. And the result is to achieving greater success in preventing bio fouling by attaching silver nanoparticles to desalination membranes (Nya et al., 2015). This current research was carried out utilizing tree barks and testing their efficacy against the fouling organism, especially against the organisms responsible for biofilm formation. Tree barks are available in the environment and their usage is very low when compared to the other

parts of the plant. All the varieties of trees have unique features. Stem, leaves, fruits, seeds are used for various research purpose.

The bark of the tree is similar to that our human skin which will prevent the tree from any damages. The present invention provides safe paint and coating composition comprising at least one environmentally acceptable phytochemical but not limited to capsicum, grape fruit seed extract and menthol suitable for use in preventing the colonization of various species. The composition of the invention will have to control the release of certain antioxidants, oxidizers or photochemical agents to promote sustained release of anti-colonization agents to control fouling. Phytochemicals are known which have broad activity on inhibiting or preventing the growth of widespread microbes, as well as exhibiting efficacy against range of potential marine fouling organisms like algae, barnacles etc., Capsicum at high pungency level are added to paints will prevent the fouling of the ship. However while showing efficacy against hard fouling by barnacles and mussels but limited effect in controlling soft fouling by algae. Another aspect of the invention on marine paints is that the phytochemical compounds like alkyl, polyvinyl acetate emulsion, polyvinyl chloride emulsion, water based epoxy resin, acrylic resin, copolymers and synthetic composites.

There are many other plants which have promising antiseptic, anti-inflammatory, antibiotic and anti-diabetic activities. Such plants namely neem**,** tamarind, mulveli , drumstick were studied for their anti-fouling properties and the results were tabulated.

MATERIALS AND METHODS

a. Tree Barks

Drumstick (*Moringaoleifera*)

Neem (*Azadirachtaindica*)

Mulveli or Mesquite (*Prosopisjuliflora*)

Tamarind (*Tamarindusindicus*)

b. Steel plate

c. Ethyl acetate, water

d. *Pseudomonas aeruginosa*, *Bacillus subtilis* are the strains ordered from MTCC.

Sample Collection

The samples are collected from the village of pakkam at Chennai

Drying

The collected samples were shade dried for one week. After shade drying, the samples are made into a powder using pestle and mortar.

EXTRACTION PROCESS

The sample extraction was carried out using soxhlet Apparatus By taking 20 grams of sample with a suitable solvent. The Apparatus run for about 6 cycles then extract was obtained and stored refrigerator for future use.

PHYTOCHEMICAL SCREENING

Test for Tannin

Take 1ml of tree bark extract and then add 1ml of 5% of ferric chloride. Formation of Greenish black colour indicates the presence of Tannin.

Test for saponin

Take 1ml of tree bark extract and then add lml of distilled water. Keep the mixture in the shaker for about 15minutes. 0.5 -1cm layer of foam will be formed. The foam formation indicates the presence of saponin.

Test for flavanoids

Take lml of each bark extract and add lml of 2N NaoH to the Mixture. Appearance of yellow colour indicates the presence of flavanoids

Test for Quinones

Take 1ml of each bark extract and add 1ml of concentration H_2SO_4. Formation of red colour indicates the presence of quinones.

Test for Glycosides

Take 1ml of each bark extract and add 1ml of chloroform along with this mixture add 10% Ammonium Solution.

Test for Cardioglycosides

Take 1ml of each bark extract and add 1ml of glarial acetic acid to this mixture add 1ml of Concenration H_2SO_4. Brown ring will be formed.

Test for Terpenoids

Take 1ml of each bark extract and add 1ml of chloroform and then add 4-5 drops of concentration H_2SO_4. Formation of Reddish brown colour indicates the presence of Terpenoids.

Test for phenol

Add 1ml of each bark extract and add 1ml of Na2co3 and then 1ml of Folin's reagent. Formation of Blue/green will indicate the presence of phenol.

Test for Coumarins

Take 1ml of each bark extract and add 1ml of chloroform 10% NaoH . Formation of yellow colour indicates the presence of coumarins.

Test for Steroids

Take 1ml of each bark extract and then add 1ml of chloroform and add 1ml of H_2SO_4. Reddish brown interface indicates the presence of steroids.

TERPENOIDS

Researchers found that Terpenoids have the ability to resist the formation of Biofilm. From the phytochemical screening *Moringa oleifera* and *Prosopisjuliflora* will have the Active constituent Terpenoids. So we choose the above two species for testing the bio film formation. Generally terpenoids also called isoprenoids , are a large and diverse class of naturally occurring organic chemicals similar to terpenes derived from 5-larbon isoprene units assembled and modified in thousands of ways. Plant terpenoids are used extensively for aromatic qualities and play a role in traditional herabal remedies. Terpenoids have different classes. Some of the terpenoids variety will inhibit the bio film formation. They are Linalool, nerol, isopulegol, menthol, carvone, thujone and farmesol exhibited bio film -specific activity.

Antimicrobial activity of *Moringa oleifrea* and *Prosopis juliflora*

Antimicrobial screening (Agar disc diffusion)

This method is suitable for organisms that grow rapidly over night at 35^{o} C-37^{o}C.

Medium

Nutrient agar plates were prepared with a uniform thickness of approximately 4mm and agar is allowed to set at ambient temperature to solidify.

Inoculum

Bacterial strains were selected for antimicrobial study. *Pseudomonas aeruginosa*, *Bacillus subtilis* are the bacterial strains used against bio film formation.

Disc diffusion method

A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube so as to express the excess fluid. The surface of nutrient Agar plate was inoculated with the swab. To ensure that the growth is uniform and confluent (or semi confluent) the swab is passed three times over the entire surface, by repeating the procedure, taking care the second and third time to turn the plate through 30µl each bark extract and which were prepared using ethyl acetate: Standard disc of Streptomycin (10µg/disc) and Tetracycline (30µg/disc) (Himedia), 6 mm in diameter were used as positive control and the solvent used for preparing extract was used as negative control. The plates were incubated overnight at 37° C for 18-24 hours. Antimicrobial activity was evaluated by measuring zone of inhibition by using Hi Media zone scale.

Agar well diffusion method

Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards. The tree bark extracts were tested on nutrient agar plates to detect the presence of antibacterial activity. Prior to streaking the plates with bacteria, 5 mm diameter wells were punched into the medium using a sterile borer. Asterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculum.

The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture. 30 µL aliquots of each test extract were dispensed into each well after the inoculation of the plates with bacteria. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with parafilm, labelled, and placed in an incubator set to 37°C. After 24 hours of incubation, each

plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimetres. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments.

GC-MS analysis for the extract of *Moringa oleifrea* and *Prosopis juliflora*

Procedure for the GC-MS analysis

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m All tech EC-5 column (250µ I.D, 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to10:1. The oven temperature program was programmed to start at 35oC, hold for 2 minutes, then ramp at 20oC per minute to 300oC and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrometry

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-20001 software was used for all analyses. Low resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Mass spectrometry library search

Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

RESULT AND DISCUSSION

Phytochemical screening of aqueous bark extract of various trees.

Phytochemical screenings of extracts were performed using standard procedures. From the phytochemical screening few active constituents present in the extracts were identified. In *Moringaoleifera* aqueous extract, Terpenoid content is high when compared to other extracts. Terpenoid has the ability to resist the formation of bio film when compared to the other constituents (Table 1).

Phytochemical screening of ethyl acetate EXTRACT

Using ethyl acetate as a solvent tree barks were tested for the phytochemical analysis . *Prosopis juliflora* will have active constituents of terpenoids, flavanoids and quinones. (Table 2)

TABLE 1:PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACTS

CONSITUENTS	*Moringaoleifera*	*Azadirachtaindica*	*Tamarindusindicus*	*Prosopisjuliflora*
Tannins	-	-	-	-
Saponin	+	-	-	+
Flavanoids	+	-	+	-
Quinones	+	+	-	+
Glycosides	-	-	-	-
Cardioglycosides	+	-	-	-
Terpenoids	+	-	-	-
Phenol	+	+	+	+
Steroids	+	-	-	+

TABLE 2: PHYTOCHEMICAL SCREENING OF ETHYL ACETATE

CONSITUENTS	*Moringaoleifera*	*Azadirachtaindica*	*Tamarindusindicus*	*Prosopisjuliflora*
Tannins	-	-	-	-
Saponin	-	-	-	-
Flavanoids	+	-	+	+
Quinones	+	+	+	+
Glycosides	-	-	-	-
Cardioglycosides	+	+	-	-
Terpenoids	-	+	-	+
Phenol	-	+	+	+
Steroids	+	-	+	+

ANTI-MICROBIAL ACTIVITY:

Antimicrobial activity of stems, roots, leaves of drumstick were performed. The root extracts of *Moringa oleifera* showed varying antimicrobial activity against tested clinical isolates with inhibition zone range of 19-35mm. Acetone extract yielded very good antibacterial activity by inhibiting 66 % isolates of *K. pneumoniae*, 80% isolates of *S. aureus*, 100% isolates of *P. aeruginosa*, S. *Pneumonia* and 86% isolates of *E. coli*.Methanol extract on the other hand inhibited 89% and 47% isolates of S. *Pneumonia* and *P. Aeruginosa* respectively.

Antimicrobial activity of the bark extract of *Moringa oleifera* showed varying activity against tested with the zone range of 24mm.The other parts of the *Moringa oleifera* like leaves, stems, fruits will show their activity against the bacterial strain. The bark extract of this tree show their activity against bio film formation.(Table 3)

Antimicrobial activity of the bark extract of *Prosopis juliflora* showed varying activity against tested with the zone range of 20mm.The bark extract of this tree also show their activity against bio film formation

TABLE 3:ZONE OF INHIBITION OF *MORINGA OLEIFERA* AND ETHYL ACETATE:

Name of the organisms	For 20µl extract(mm)	For 40µl Extract(mm)	For 50µl Extract(mm)	Positive control ciprofloxacin
Psedomonas aeruginosa.	11	15	24	30
Bacillus subtilis	10	13	20	28

FIGURE 1: ANTI-MICROBIAL ACTIVITY OF ETHYL ACETATE

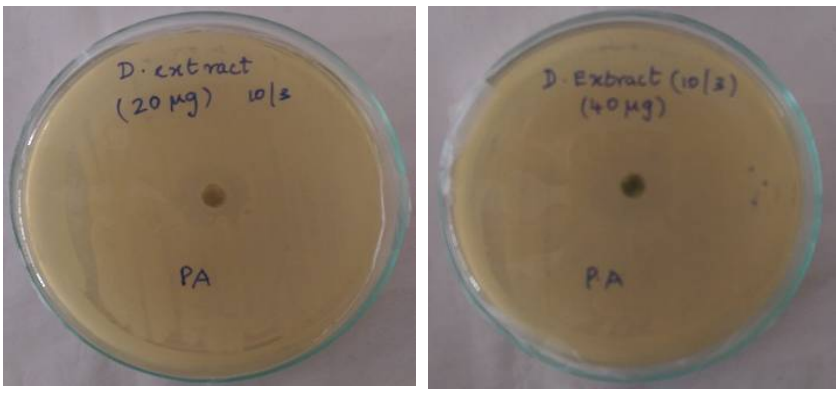

FIGURE 2: ANTIMICROBIAL ACTIVITY OF AQUEOUS BARK

Extract of *Moringa oleifera* at different concentrations

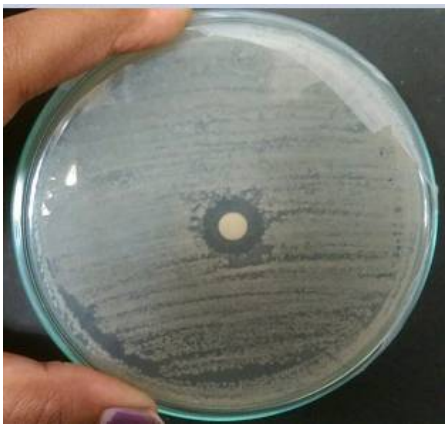

FIGURE 3:ANTIMICROBIAL ACTIVITY OF ETHYL ACETATEEXTRACT OF*prosopis juliflora*

GC-MS RESULTS:

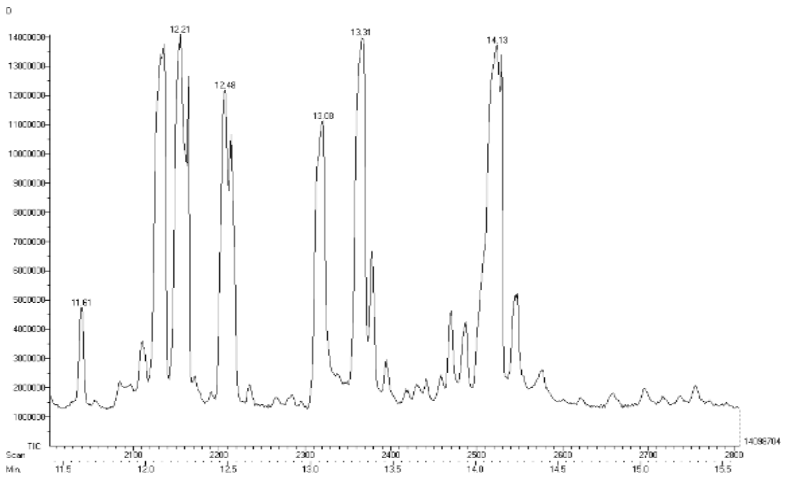

FIGURE4 : The peak area percentage and peak area coverage of the Sample *Moringa oleifera* is given below.

TABLE 4 : SHOWS THE PEAK AREA,COMPOUND NAME AND PEAK %.

Peak no.	RT(min.)	Compound name	Peak area	Peak area(%)
1.	11.61	a-Caryophyllene	4751504	6.80
2.	12.21	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8aoctahydronaphthalene	14098704	20.18
3.	12.48	2-Isopropenyl-4,4,7a-trimethyl-2,4,5,6,7,7ahexahydro-benofuran-6-ol	12178544	17.43
4.	13.08	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-[Z]]-	11127936	15.93
5.	13.31	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethylene-,[1ar-[1aa,4aa,7a,7aa,7ba]]-	13989920	20.02
6.	14.13	1-Naphtalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-[1-methylethyl]-,[1R-[1a,4a,4aa,8aa]]-	13726576	19.64
		Total	69873184	100.00

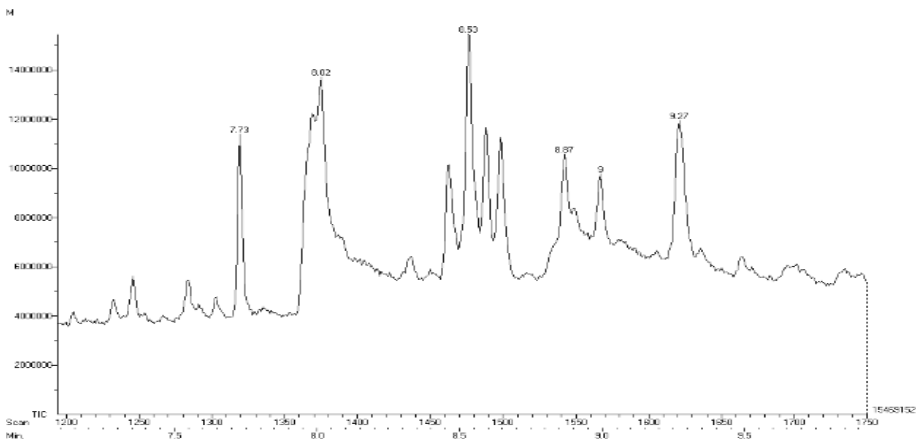

FIGURE 5:The peak area percentage and peak area coverage of the Sample M is given below.(Table

TABLE 5: SHOWS THE PEAK AREA,COMPOUND NAME ANDPEAK(%).

Peak No.	RT (Min.)	COMPOUND NAME	Peak Area	Peak %
1.	7.73	16-Hexadecanoylhydrazide	11388336	15.63
2.	8.02	Estra-1,3,5[10]-trien-17a-ol	13716832	18.82
3.	8.53	Oleic acid	15469152	21.23
4.	8.57	Dasycarpidan-1-methanol, acetate [ester]	10568528	14.50
5.	9	Ethyl 2-acetylamino-3-[2-oxo-piperidin-1-yl]propanoate	9783712	13.43
6.	9.27	1,7,7-Trimethyl-3-phenethylidenebicyclo[2.2.1]heptan-2-one	11939600	16.39
		Total	72866160	100.00

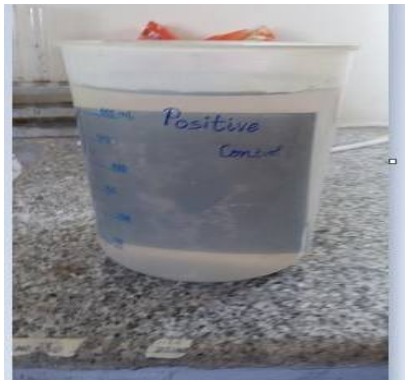

FIGURE 6: SHOWS THE STAINLESS STEEL PLATE WITHOUT BIOFILM FORMATION.

FIGURE 7: SHOWS STAINLESS STEEL PLATE AFTER 24 HOURS OF BIOFILM FORMATION:

FIGURE 8: THE STAINLESS STEEL PLATE COATED WITH EXTRACT OF MORINGA OLEIFERA AND PAINT WITHOUT BIOFILM FORMATION.

The compounds and their activities present in the *Moringaoleifera* extract was discussed below:

Humulene, also known as α-humulene or α-caryophyllene, is a naturally occurring monocyclic sesquiterpene (C15H24). Humulene is an isomer of β-caryophyllene, and the two are often found together as a mixture in many aromatic plants. Humulene has been found to produce anti-inflammatory effects.

(-)-α-Selinene is the common name of 2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8aoctahydronaphthalene.

The molecular name of the 2-Isopropenyl-4,4,7a-trimethyl2,4,5,6,7,7ahexahydro-benofuran-6-ol is c14H2212.

Spathulenol is the common name used for the 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethylene-,[1ar-[1aa,4aa,7a,7aa,7ba]]-(Table 4)

CONCLUSION:

The experiment was carried out with the extract of tree bark to check the formation of biofilm in the stainless steel plate (Figure 6,7). Out of four samples of tree barks two samples were choosen form the result of antimicrobial activity. The two bark extracts were mixed with paint and coated on the steel plate.The result will show the inhibiting capability of the extracts against biofilm formation (Figure 8). The non-target marine organisms will not get affected by the paint with tree bark extract. Compared to the other antifouling paints the chemical compound activity will be very low when the paint is mixed with the bark extract of above studied trees.

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