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Bio potentials of marine natural products to combat bio fouling and corrosion

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Abstract: Biofouling causes serious problems for marine industries and navies around the world. It requires a solution that doesn't give trouble to our environment. In recent years the use of natural products to control biofouling has been gaining importance. In this respect, phytochemical study indicated that tested seaweeds and mangrove plant showed positive results for the presence of Saponins, Glycosides, Steroid, and Terpenoid. The corrosion inhibition activity is mainly correlated with the major active functional groups (hydroxyl, amino, carbonyl and phosphoryl functionalities, aliphatic (fatty acids), NH2 (amide I & II)) of the extracts. The bonds such as O-H stretch, H-bonded, C-H stretch, -C=C- stretch, C-O stretch, are principally involved in inhibition activity found in all the extracts. Also, the organic extracts of five marine algae and one species of mangrove were screened for their antibacterial property. Among the five seaweeds studied *Gracilaria edulis* showed more antibacterial activity particularly against seawater bacteria (15mm). *Enteromorpha intestinalis* (*E. intestinalis*), *Kappaphycus alvarezii* (*K. alvarezii*), *Gracilaria edulis* (*G. edulis*) and *Ulva lactuca* (*U.lactuca*)showed appreciable level of inhibition zone against *Corynebacterium* (11mm), *Flavobacterium* (10 mm), *Micrococcus sp*.(10 mm) and *Achromobacter* (10 mm) respectively. Among the five seaweeds, *U. lactuca* exhibited a maximum inhibition of 88.5%.The present study suggests that *E. intestinalis, G. edulis* and *U.lactuca and A. marina* can be considered as potential natural sources for developing anti- fouling and anticorrosive coatings.

Key Words: *Biofouling, corrosion, Antifouling, Gracilaria edulis, Phytochemicals.*

1. INTRODUCTION:

The undesirable phenomenon of adherence and accumulation of biotic deposits on submerged artificial surface structures such as ship hulls, jetty pilings, aquaculture net cages, seawater pipes are termed as Biofouling. This accumulation or incrustation consists of a film composed of microorganisms affixed to a polymeric matrix created by themselves, where inorganic particles like salts and/or corrosive products may arrive and be retained as a consequence of other types of fouling that develop in the course of the process [(1)].This biofilm can begin the accumulation of macro-organisms (macro fouling).Biofouling is made up of hundreds of species, such as bacteria, protozoan, seaweeds, mollusks, bryozoans, cirripeds, polychaetes, ascidians, hydrozoans, and so on. These organisms adhere themselves to the substrate, developing a fast growth rate and great reproductive potential.

Biofouling, accelerates the processes of corrosion of manmade materials and causes break downs in the performance of the structures [(1,2)].This damage takes place on movable and stationary structures such as boats, petroliferous or gas platforms, oceanographic investigation implements, thermal energy conversion plants, and subaqueous wounding equipment. It also damages maritime cultivation facilities (aquariums, cages, conduits, and pumps), as well as their cultivated organisms. In ships, biofouling increases the friction between the hull and the water, which results in an increase in fuel consumption which can lead to enormous economic losses. Anti-fouling is the process of removing the accumulation, or preventing its accumulation. In industrial processes bio-dispersants can be used to control biofouling. Antifouling paints are paints that we apply on the underwater hulls of ships and boats. Antifouling paints function by slowly releasing toxins into the lamellar sub layer of the vessel, protecting and preventing settlements of fouling organisms. Generally, the type of toxins used and the way these toxins are released differentiates the various classes of antifouling paints. The most common types of toxins used in antifouling paints are copper and tributyltin moiety (TBT) [(3)] .Many types of coatings, however, have been found to be toxic to marine organisms. For example, extremely low concentrations of [TBT (1,4)], the most commonly used anti-fouling agent, cause defective shell growth in the oyster *Crassostrea gigas* and development of male characteristics in female genitalia in the dog whelk *Nucella lapillus* [(5)].Hence The International Organisation (IMO) and Marine Environmental Protection

Committee (MEPC) has banned the usage of TBT or other substances containing tin as biocides in antifouling paints, since January 2008. The ban of organotins such as TBT and triphenyltin (TPT), and other toxic biocides in marine coatings is a severe problem for the shipping industry; it presents a major challenge for the producers of coatings to develop alternative technologies to prevent fouling on ship hulls. Therefore, there is a need for the development of ‘‘environmentally-friendly’’ nontoxic anti-fouling formulations.

Natural products from marine organisms have been suggested as replacements for the chemicals commonly used in antifouling coatings. All over the world efforts are oriented towards isolation of eco-friendly antifouling toxins from marine plants and organisms [(6)]. Consequently number of compounds having antifouling properties have been identified from marine plants and organisms by number of workers in the past. However, their efficiencies in combating the fouling in the real system i.e. in the natural seawaters have not been adequately demonstrated. In line with the fore going account, in the present study an attempt has been made to investigate the bio potentials of certain seaweeds and mangrove plants with the following objectives.

2. MATERIALS AND METHODS:

Five different stains were isolated and characterized using biochemical tests and found to be *Pseudomonas sp, Achromobacter sp, Corynebacterium sp, Flavobacterium sp* and *Micrococci sp.* The biofilm samples from various substrates exposed to natural seawater below the CECRI’s offshore Platform at Tuticorin, were collected by scrap method using sterile brush and immediately transferred to a culture tube containing 10 ml of sterile seawater. And all the samples were serially diluted using sterile seawater. Bacterial enumeration was done by pour plate method [(7)]. Different tests were performed for Characterization and identification of Bacteria as per the keys given in the Bergey’s manual.

2.1. Collection and extraction preparation of seaweeds and Mangrove plant

Live and healthy samples of brown and green seaweeds were collected from Tuticorin coast (08° 46′25.15″ N lat., 78° 11′16.05″ E long.) and identified to be *K. alvarezii, U. lactuca, E.intestinalis, P. boergesenii* and *G. edulis*. The mangrove plant was collected from back waters near Tuticorin Thermal Power Station and identified to be *A. marina*. Seaweeds were thoroughly washed and allowed to air dry at room temperature and then pulverized. 50 g of the sample was taken in a Soxhlet apparatus for the crude extraction**,** with about 100 ml of diethyl ether as solvent. This was repeated with all the samples.

3. METHODS:

Phytochemicals of the natural products were analyzed by the method of Yadav [(8)]. Functional groups were characterized using FT-IR (Nexus 670-ThermoElectron corporation make by method of Ramya *et al* [(9)]. Disc diffusion method under by Wong [(10)] was employed for the study of antibacterial activity employing biofilm forming bacteria on basis of Govindaraju [(11).] Study of antifouling activity was followed by Fusetani [(12)]. Corrosion inhibition Study by the method of Marko [(13)] .Coupon Preparation with the help of Kingsley [(14)] and Weight loss measurements - Mild steel specimens were immersed in triplicate in 100 ml of the test (1 N HCl) with and without addition of natural product extract of different. After the desirable interval periods, the test specimens were removed and their weight losses were determined using [(14)].

4. RESULTS AND DISCUSSION:

4.1. Phyto-chemical analysis of seaweeds and mangrove plant:

Results of phytochemical analysis of seaweeds and mangrove leaves were given in Table 1. The tested seaweeds and mangrove plant showed positive results for the presence of Saponins, Glycosides, Steroid, and Terpenoid. However mangrove leafs was found to contain almost all the phytochemicals tested excepting flavonoids and glycosides. Secondary metabolites of marine organisms play a vital role to control the fouling [(15)]. More phytochemicals may be responsible for the observed highest inhibition efficiency. Synergism effects with other components of the plants, especially the tannins, may increase the inhibition efficiency to an appreciable extent [(16)]. The use of modified tannin from black wattle tree used as an antifouling pigment and it’s formulated in antifouling coating exposure in a marine environment and can eliminate the release of metals and other toxic biocides to the marine environment [(17)]. Steroids indicating that it might be a nontoxic antifoulant [(18)], Phenol, glycoside, saponin, and terpinoids possess the ability to disrupt biofilms [(19)].

4.2. FT-IR study:

FT-IR spectral studies were carried out for seaweeds, and mangrove extracts and their band spectra and corresponding functional groups are given in Tables 2-7 and Graph.1-6. Absorbance frequencies for *k. alvarezii* show the presence of primary amines; aliphatic groups (RCH2- NH2) at 3327.3 and amides group C-C (=O) $–NH_2$) at

1634.9. Functional groups of *U.lactuca* recorded by FT-IR spectrum are of secondary aliphatic alcohols at 3633.5, primary amides (C-C (=O)-) at the peak 3349.4, aromatic-CH_3 groups at the peak 2853.5.Carbonyl bands like acid anhydrides (–CO-O-CO-) could be noticed at the peak 1814.5.Peak 1161.5 and 1376.5 indicates the presence of isopropyl group and aliphatic-CH3. *E.intestinalis* show peak at 3323.6 indicating the presence of secondary amines, peak 2361.6 indicates phosphorus compounds; peak 1353.3 corresponds to aromatic nitrogen. The presence of alkanes and aliphatic-CH3 could be evident from the peaks at 1442.2 and 1473.1respectively. The FT-IR spectrum of *P.boergesenii* extract shows the absorption bands at 1536, 2836.6 and 1009.9 which correspond to nitrogen compounds like amides -C (=O)-NHR, phenols and primary aliphatic alcohols respectively. The bands for *G.edulis* could be attributed to the primary aliphatic alcohols, pyrroles at 3633.6 and 3349.3 respectively. Aromatic-CH_3 groups with symmetric stretches were found at 2921.6 and 2853. Peak 1814.5 shows the presence of vinyl group ($RHC=CH_2$), the presence of nitrogen atoms could be seen at the peak 1525.8 and the peaks 1188.6 and 968 indicate the presence of Gem-Dimethyl group and (RHC=CHR) trans-dialkyl groups respectively. For the mangrove plant *A.marina* FT-IR band absorbance frequency at 3324.7 indicates the presence of secondary amines with aliphatic compounds (R-NHR) with N-H stretching. The peak at 2360.3 with B-H stretch could be linked to boron compounds. Peak at 1643.4 attributes to the amides (C-C (=O)-NH_2) group. The inhibition activity is mainly correlated with the major active functional groups (hydroxyl, amino, carbonyl and phosphoryl functionalities, aliphatic (fatty acids), NH_2 (amide I & II)) of the extracts. The bonds such as O-H stretch, H-bonded, C-H stretch, -C=C- stretch, C-O stretch, are principally involved in inhibition activity and mainly found in all the extracts. Hence, they can be considered as potential natural sources of bioactive metabolites.[20, 21].

4.3. Antibacterial activity of seaweeds and Mangrove plant:

In the present study antibacterial activities of five seaweed extracts and one mangrove plant were studied against the pure cultures of five bacterial strains isolated from marine biofilm viz., *Pseudomonas sp., Acromobacter sp., Corynebacterium sp., Flavobacterium sp., and Micrococci sp.*, and also against mixed cultures of seawater bacteria. The results in terms of inhibition zone are illustrated in Table 8. The extracts of *K.alvarezii, U.lactuca, E.intestinalis, G.edulis* and showed appreciable level of inhibition zone. Pseudomonas sp, was found to be resistant to all the seaweeds extracts studied. The extract of mangrove leaves showed broad spectrum antibacterial activity with maximum inhibition zone of 17mm against Micrococcus sp. and 10 mm inhibition zone against *Pseudomonas sp.* Among the five seaweeds studied *G. edulis* showed more antibacterial activity particularly against seawater bacteria (15mm) followed by *E. intestinalis* (14 mm) , *and U.lactuca* (13 mm each).As far as the pure cultures of biofilm forming bacteria are concerned none of them are sensitive to the algal extract of *P.boergesenii.* However, the extracts of *E. intestinalis, K.alvarezii, G.edulis* and *U.lactuca* showed appreciable level of inhibition zone against *Corynebacterium* (11mm), *Flavobacterium* (11 mm), *Micrococcus sp.* (10 mm) and *Achromobacter* (10 mm) respectively. *Pseudomonas sp*, was found to be resistant to all the seaweeds extracts studied. On contrary to the seaweeds, the extract of mangrove leaves showed broad spectrum antibacterial activity with maximum inhibition zone of 17mm against Micrococcus sp. and 10 mm inhibition zone against Pseudomonas sp. The observed variation in the inhibitory levels of marine natural products, in the present study, could be attributed to the varying functional groups in the natural products [6, 22]. Most of the algal extracts similar to the present study, the algal extracts and mangrove leaf extract showed antibacterial activity against the biofilm bacteria tested [23]. In the present study, the *A. marina* extract showed broad spectrum antibacterial activity against all the 5 biofilm forming bacterial isolates with relatively maximum inhibition zone. Similar observation was earlier reported [20]. According to them *A. marina* limited the growth of *Flavobacterium sp.* (16 mm) and *Bacillus sp.* (20 mm) and the extracts of *Rhizosphere mucronata* limited the growth of *Flavobacterium sp.* (18 mm) and *Bacillus sp.* (18 mm). While comparing the inhibition activities of all the extracts, mangrove plants extracts had higher inhibiting activity against primary biofilm forming bacteria than seaweeds and seagrasses. Hence the present work suggests that the marine seaweeds and mangrove plant are potential sources of antibacterial compounds and may be further investigated with various fractions of the extracts.

4.4. Natural products activity on fouling inhibition by Epi- fluorescence and Crystal violet: Staining Method

Epi- fluorescence results revealed lesser bacterial population in the system treated with mangrove extract when compared to that of the control system. Mangrove plant extract shows maximum inhibition for *micrococcus sp.*, and *pseudomonas sp*., whereas for *corynebacterium sp.*, it showed less or no inhibitory action .Seaweed extracts of *P.boergesenii* and *U.lactuca* showed relatively maximum effect. *K. alvarezii* showed bactericidal effect, this can be observed by the formation of red color in epi-fluorescence images. *E. intestinalis* and *G.edulis* showed least inhibitory effect. Based on the principle of crystal violet, the dead cell lack to absorb the dye whereas the attached and living cells appear in violet color even after the application of decolorized. This staining results support the results of Epi-

fluorescence studies. Maximum absorption of dye was observed in the control tube, where as the tubes that were used for the biofilm development in the presence of natural product extracts shown almost negligible or no absorption of dye. The variation in the effects of algal extracts suggests that they are not simply functioning as broad spectrum toxins against marine bacteria; rather they appear to have specific activities against one or several biofilm bacteria. The extracts of mangroves were reported to have antifouling properties [20, 24, 25]. In the present study, the sensitivity of bacterial isolates, to all the extracts could be attributed to the presence of common bioactive compounds that had inhibitory effects on the microorganism as opined [25].The overall antibiofilm metabolites assessed from the present results indicates the availability of active constituents in the extractions which showed better antimicrobial activity against micro-fouling bacteria. Hence, they can be considered as potential natural sources of bioactive metabolites acting as leading anti-biofilm [20, 21].

4.5. Corrosion inhibition study:

The corrosion inhibition efficiencies of the natural product extracts in 1N HCl for mild steel corrosion for 1 hr duration is shown Graph 1. The results revealed that all the six natural products inhibit corrosion of mild steel in 1 N HCl. Among them the seaweeds, *U.lactuca* exhibited a maximum inhibition of 88.5 % followed by *G.edulis* (88.18%), *E.intestinalis* sp. (86.23%), *and A.marina (76.68%).The* other two seaweeds *K. alvarezii* and *P.boergesenii* sp. showed marginal corrosion inhibition. This implies that the corrosion inhibitors adhere on the surface and so give more efficient inhibition effects to decrease the corrosion rate [26].

5. CONCLUSIONS:

The extracts studied showed good inhibition efficiency. Among the five seaweeds, *U.lactuca* exhibited a maximum inhibition of 88.5%.The present study suggests that *E. intestinalis, G. edulis* and *U. lactuca and A. marina* can be considered as potential natural sources for developing anti- fouling and anticorrosive coatings. The bacterial population in biofilm was found drastically decreased. From this study clearly shows an enhanced important source of natural antibacterial, antifouling as well as anticorrosion activity present in the experimental samples.

Conflict of interest

We declare that we have no conflict of interest.

Table -1: Phytochemical analysis of seaweeds and mangrove plant

Sample	Flavonoids	Phenols	Tannins	Saponin	Glycosides	Steroids	Terpenoids
K. alvarezii	-	-	-	+	+	+	+
U.lactuca	-	-	-	+	+	+	+
E.intestinalis	-	-	-	+	+	+	+
P.boergesenii	-	-	-	+	+	+	+
G.edulis	-	-	-	+	+	+	+
A.marina	-	+	+	+	-	+	+

Table-2 Identification of functional groups of *kappaphycus alvarezii* by FT-IR Analysis

Sample	Frequency /cm	Functional group	Remarks
k.alvarezii	3327.3	Primary amines:Aliphatic,RCH2- NH2	Symmetric NH2 strech;dilute solution
	1634.9	Primary amines:Amides, C-C (=O) –NH_2	NH_2 scissors, Nujol

Table -3 Identification of functional groups of *Ulva lactuca* by FT-IR Analysis			
Sample	**Frequency /cm**	**Functional group**	**Remarks**
U. lactuca	3633.5	Secondary aliphatic alcohols	Free OH stretch CCl_4
	3349.4	Primary amides	Amides, C-C(=O)-NH_2
	2921.9	Aromatic-CH_3	-
	2853.5	-CH_2-	Symmetric stretch
	2361.7	Tertiary amine salts NH^+-	Multi bands
	1814.5	Carbonyl Bands: Acid anhydrides –CO-O-CO- Saturated	Two bands usually separated by about 60 cm^{-1}.The higher frequency band is more intense in acyclic anhydrates,and the lower frequency band is more intence in cyclic anhydrides.
	1442.9	-CH_2-	Symmetric stretch
	1376.5	Aliphatic –CH_3	-
	1161.5	Isopropyl group	May shift to higher frequency if another branched carbon is adjacent

Table -4 Identification of functional groups of *Enteromorpha intestinalis* by FT-IR by Analysis			
E.intestinalis	3323.6	Secondary amines:Aliphatic, R-HR	N-H stretching, liquid
	2361.6	Phosphorus compounds:PH and PH_2	Sharp stretching bands
	1632.0	Conjugation with a nitrite group	C=C streching
	1473.1	Aliphatic –CH_3	Asymmetric bending
	1442.2	Alkanes:-CH2-	Scissoring
	1353.3	Nitro group:Aromatic	NO_2 symmetric strech

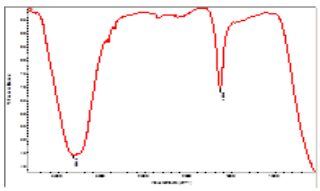
Table -5 Identification of functional groups of *Padina boergesenii* by FT-IR Analysis			
Sample	**Frequency /cm**	Functional group	**Remarks**
P. boergesenii	2946.9	-CH_2-	Asymmetric stretch
	2836.6	Phenols:Chelation	Broad; occasionally weak; the lower the frequency, the stronger the intramolecular bond
	2361.9	-BH_2	B-H stress, usually a doubler also B-H
	1703.0	-C(=O)-CH=CH_2	C=O stretching
	1608.2	Aromatic, Aryl-NH_2	NH_2 scissors
	1536	Amides,-C(=O)-NHR	C-N-H bend,trans amide II, dilute solution
	1444	Alkanes:-CH_2-	Scissoring
	1322.3	Amide, -C(=O)-NHR	Cis C-N stretch;for trans see amide II and amide III band under N-H bend
	1077.8	Boron Compounds:B-Cl	Plus other bands at lower frequencies
	1009.9	Primary aliphatic alcohols	Out-of-phase C-C-O strech
	869.7	Hetero aromatic Bands:Pentasubstitution	Diagnostic band
	755.3	Alkenes:RHC=CHR trans-dialkyl	CH wag

Table -6 Identification of functional groups of *G edulis* by FT-IR Analysis			
Sample	Frequency /cm	Functional group	Remarks
G.edulis	3633.6	Primary aliphatic alcohols	Free OH stretch; only in very dilute, nonpolar

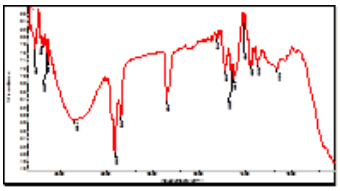
			solvents
	3349.3	PYRROLES:3-Substituted	Bonded N-H stretch
	2921.6	Aromatic-CH_3	-
	2853	Aromatic-CH_3	Symmentric stretch
	2361.6	-BH_2	B-H stress, usually a doubler also B-H
	1814.5	RHC=CH_2 vinyl	=CH_2 Asymmentric stretch
	1717.8	Ayrl and α,βunsaturated,α, β, α^1,β^1, unsaturated and diaryl	-
	1631.9	Tertiary	Since H-Bonding is absent solid and solution spectra are same
	1525.8	Amide,-C(=0)-NHR	C-N-H bend, trans amide II dilute solution
	1459.3	Sulfuryl halides X-SO_2-X	Strength:F (w) and Cl (s)
	1376	Sulfonamides-SO_2-N	-
	1188.6	Gem-Dimethyl group(in alkanes)	-
	968	RHC=CHR trans-dialkyl	CH wag

Table -7 Identification of functional groups of *A. marina* by FT-IR Analysis

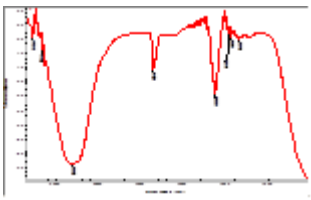
Sample	Frequency /cm	Functional group	Remarks
A.marina	3324.7	Secondary amines: Aliphatic, R-NHR	N-H stretching, liquid; 3360-3310/cm in dilute solution.
	2360.3	Boron compounds:-BH_2	B-H stretch, usually a doublet; also B-H stretch for =B-H
	1634.4	Amides, C-C(=O)-NH_2	NH_2 scissors, Nujol



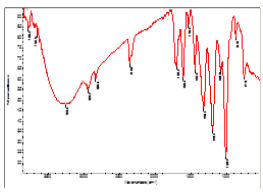
Graph-1: *K.alvarezii* FT-IR graphical images



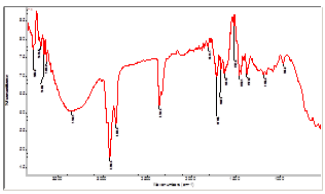
Graph-2: U.lactuca in FT-IR graphical images



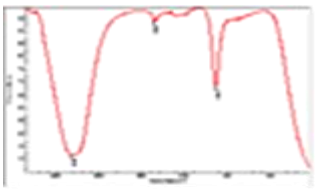
Graph-3: *E.intestinalis* in FT-IR graphical images



Graph-4: *P.boergesenii* in FT-IR graphical images



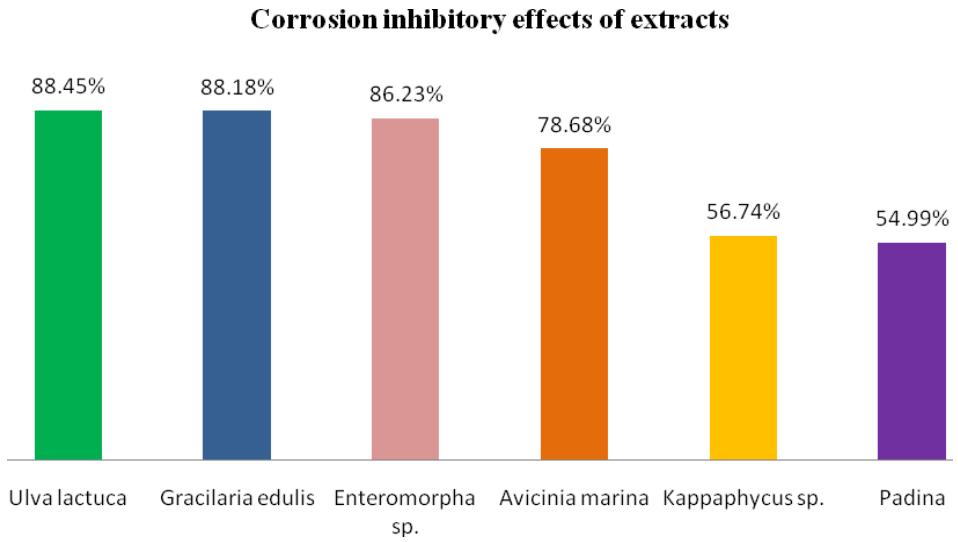
Graph-5: *G.edulis* FT-IR graphical images



Graph-6: *A.marina* FT-IR graphical images

Table-8: Antibacterial activity of mangrove plant and seaweed extract

	Zone of inhibition in (mm)					
SAMPLES	***Pseudo monas***	***Micro coccus***	***Coryne bacterium***	***Flavo bacterium***	***Achromo bactor***	**Mixed culture**
K.alvarezii	-	-	2	11	-	7
U.lactuca	-	4	2	2	10	13
E. intestinalis	-	-	11	4	2	14
P.boergesenii	-	-	-	-	-	7
G.edulis	-	10	4	5	4	15
A.marina	10	17	5	4	5	8



Graph-7: Corrosion inhibition efficiencies of natural products in 1 N HCl for Mild steel

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