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Production of Antifouling Paints' using Environmentally Safe Algal Extracts on Laboratory Scale

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ABSTRACT

Fouling is a result of the accumulation of bacterial growth, algae and sessile invertebrates on both natural and manmade submerged surface. To combat fouling ships must constantly undergo cleaning up and maintenance processes. This work aimed to evaluate new biocide antifouling paints produced from marine algae, and study their suppressive effect on slime film forming bacteria. In addition, the effect of the leached components from these wood coated surfaces containing algae on the characteristics of the contact seawater medium was investigated compared with two commercial marine paints. To evaluate the suppressive effect of algae containing coatings, extraction of the new biocide antifouling paints were done by mixing different ingredients % of binder, pigment, filler, stabilizer and solvent. The obtained paints were durable, long lasting with no cracking formed before their incorporation with algae. The paint formulations were applied to wood panels and immersed in sterile glass beakers filled with seawater medium collected from the Eastern Harbour, Alexandria, Egypt. The physicochemical parameters (temperature, salinity, pH, dissolved oxygen and nutrient salts) of seawater samples around the tested panels' were measured after four weeks of immersion and the microbiological examination for the panels were measured after two and four weeks of immersion. Using of algae in the dry paint film leads to highest suppressive effect (%). The tested *Ulva fasciata*, *Corallina mediterranea* and *Codium Tomentosum* were having the highest suppressive effect 100, 99.6 and 99.5%, respectively. These results matched with the characterization by gas chromatography-mass spectrophotometer (GC-MS) and Fourier transform infrared spectroscopy (FTIR).

INTRODUCTION

Fouling describes the settlement and growth of marine plants and animals on submerged structures. These structures typically include ships' hulls, piers, piling and oil rigs. Micro fouling organisms on the surface of a substratum form heterogenic biofilms.

These biofilms on artificial structures create serious problems for industries worldwide, with effects including an increase in drag force and metal corrosion as well as a reduction in heat transfer efficiency. Additionally, microorganisms produce chemical compounds that may induce or inhibit settlement and growth of other fouling organisms (Dobretsov *et al.*, 2013). Beside fouling effects on the propulsion of ships as it results in a reduction of speed by approximately 2% and increased fuel costs from 6 to 45% depending on the size of the ship, fouling introduces new species into marine environments and reduce the efficiency of underwater acoustic devices. However, the demand to develop a novel, environmentally friendly antifouling (AF) material is ever increasing, after the ban of TBT based antifouling by the International Maritime Organization (IMO) in 2008 (Kristensen *et al.*, 2008; Chapman *et al.*, 2014; Satheesh *et al.*, 2016). Marine organism-derived secondary metabolites are promising potential sources for discovering environmentally safe antifouling agents. Of the 160 potential products collated, 76% are from sponges, algae and cnidarians (Chambers *et al.*, 2006).

Marine algae are one of the largest producers of biomass in the marine environment. They produce a wide variety of chemically active metabolites in their surroundings. These active metabolites have antibacterial, antiviral, antifungal and anti-macrofouling properties, which are effective in the prevention of biofouling process (Bhadury and Wright, 2004).

The objective of the present study is to test and evaluate the antifouling activity of natural-based paints from marine algae on wood panels in order to investigate the most efficient and safe paint compared to some commercial marine paints.

MATERIALS AND METHODS

Seawater collection site and the physicochemical measurements

The seawater samples were collected from the Eastern Harbour (EH), Alexandria, Egypt during winter 2017. It characterized by eutrophic condition (Faragallah *et al.*, 2009). The physicochemical measurements of the used seawater were detected twice during this study period (zero time and after 4 weeks of experiment). The temperature, salinity, pH-value, dissolved oxygen (DO) and nutrients salts (ammonia, nitrite, nitrate, phosphate and silicate) were estimated. Water temperature was measured using an inductive portable thermometer. Salinity was measured using Salinometer model Beckman RS-10-X3 range to about 0.1 units. The pH- values of water samples were measured to about 0.1 unit in situ by using a portable pH-meter (Orion Research model 210 digital pH- meters). DO was determined according to the classical Winkler's method modified by Grasshoff (1976). Nutrients salts were determined calorimetrically according to the methods described by Parsons *et al.* (1984) and the absorbance was measured using double-beam spectrophotometer model Shimadzu UV-150-02. The values were expressed as µmol/l.

Algal materials and the extraction process

Seven algae samples were collected by hand from an exposed rocky site near the western edge of Abu Qir Bay during winter 2017. The identification of the algal species was carried out according to Chapman and Chapman (1983). The algal species were identified as Chlorophyceae; *Ulva lactuca*, *Ulva fasciata* and *Codium tomentosum,* Phaeophyceae; *Colpomenia sinuosa,* Rhodophyceae; *Pterocladia capillacea, Jania rubens* and *Corallina mediterranea*.

These samples were thoroughly washed using distilled water in the laboratory and then oven dried at 40^0C until a constant weight was obtained. These dried algal samples were grinded to powder till mesh size 50 micron in a porcelain mortar (16.0 cm), then preserved in well-sealed, labeled small plastic bags, and kept in a deep freezer at -20^0C (not more than one week) till extraction process. The extraction was performed for the seven algal species with methanol of 1:5 w/v for three times each time took 24 hrs to obtain crude methanolic extracts. The dry methanolic extracts were added to 50 ml of prepared antifouling paint formulation.

Antifouling paint formulation

The paint formulation contains: 25 gm of oil binder material, 10 gm of iron oxide, 24 gm of zinc oxide, 13 gm of complementary pigment and 38 gm of xylene. Each algal extract was added solely to this marine paint formulation. Extract concentration of each alga in paint was tabulated in Table 1.

Table 1: Algal extract concentrations in marine paint formulation (mg/ml)

Algae	Concentration of algae extract in paint
Corallina mediterranea	17
Codium tomentosum	20
Ulva lactuca	20
Ulva fasciata	20
Pterocladia capillacea	20
Jania rubens	18
Colpomenia sinuosa	20

Preparation of wood panels

Wood panels with dimensions 7×3×0.5 cm^3 were used. Their surfaces were polished using different grades of emery papers until finesse grade.

Marine paints preparation

The seven paint compositions followed by the two industrial products (Sipes Transocean Coatings Optima Antifouling 2.36 [R1] and International Interswift 6600 TBT Free Antifouling [R2]) were applied on wood panels with two successive coats by brush forming different nine coated panels. The weight of paint film on wood panels was found in Table 2.

Table 2: Weight of paint film on wood panels

Type of algae	Weight before painting (gm)	Weight after painting (gm)	Weight of paint (gm)	Weight %
Corallina mediterranea	7.6970	10.5909	2.899	27.37
Codium tomentosum	7.9803	10.1650	2.185	21.50
Ulva lactuca	7.8465	9.1062	1.260	13.84
Ulva fasciata	7.8289	9.6093	1.780	18.52
Pterocladia capillacea	7.7039	9.8510	2.147	21.79
Jania rubens	8.2317	10.4663	2.235	21.35
Colpomenia sinuosa	7.7969	10.3627	2.566	24.76
R1	7.4883	9.8201	2.332	23.75
R2	7.7250	9.7015	1.977	20.38

Antifouling experiment

The coated wood surfaces with the marine paints were hanged for each panel separately in a sterile beaker containing seawater (2 l). The immersion was applied at

laboratory during winter season. The physicochemical parameters for seawater of all beakers were determined before immersion and at the end of the experiment after 4 weeks. The microbiological organisms were gathered from the panels' surfaces after 2 and 4 weeks (Fig. 1).

Fig. 1: Zero time wood panels immersed in seawater

Microbiological examinations

The slime film formed on each tested wood panel surface 3×7 cm^2 was collected periodically along the experiment in sterile Eppendorf tubes containing 1 ml sterile seawater using sterile tooth pics. Then the aerobic bacterial count was estimated as colony forming unit per centimeter^2 (CFU/cm^2) using the pouring technique according to Chythanya and Karunasagar (2002). In which about 15 ml of melted nutrient agar at 45ºC was poured on 100 µl for each plate. Viable bacterial counts (CFU/cm^2) were estimated in duplicate as follows:

CFU/ cm^2 = Count of the viable bacteria on plate × Dilution / Surface area of the tested panel

The suppressive effect % of the bacterial count expressed as a +ve sign or the slime film accumulation % of the bacterial count expressed as -ve sign of the tested paints was calculated in relation to the slime film formed using the blank (B) (paint without biocide). These effects were estimated after two and four weeks of seawater immersion follows the modified equation from Tadros *et al.* (2009) as follows:

Suppressive effect % or Slime film accumulation %= (B-A/ B) × 100

Where B is the bacterial count on the blank wood panel and A is the bacterial count on the treated coated wood panels.

Characterization of antimicrobial bioactive compounds

Partial Characterization of methanol extracts using gas chromatography-mass spectrophotometer (GC-Ms)

The analysis was conducted using GC-Ms (Trace DSQii MS) for the potent algal extracts. It was carried out at the Marine Pollution Lab of National Institute of Oceanography and Fisheries, Alexandria, Egypt. The component(s) were identified by comparing their retention times with those of authentic samples, as well as by comparing their mass spectra with those of Wiley 275 library (Jerkovic *et al.*, 2015).

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy used to determine the vibration frequency changes in the functional groups of the potent marine algal extracts under ambient conditions. The spectra were collected using a model Broker Vertex spectrometer within the wave number of 400-4000cm^{-1}.

RESULTS AND DISCUSSION

The physicochemical parameters measurements of seawater

Corresponding to the winter season 2017, the physicochemical parameters for the wood panels after four weeks of seawater immersion on Laboratory scale are shown in Tables (3-4). These results were compared with the control (seawater medium at zero time).

Temperature

The seawater in the sampling area possesses a mild temperature (Table 3). However, little variation could be observed with a maximum value after four weeks of immersion for the coated wood panels (17.7^0C) and a minimum value of 17.4^0C during the same period.

Salinity

It can be noticed that only slight variations in the salinity were recorded for the Eastern Harbour seawater medium contained the coated wood panels (37.35-37.31). The salinity of seawater medium for wood panels coated by different algae showed lower values than their corresponding control seawater.

The pH values

The pH of wood panels coated with different algae showed mostly higher pH values than their corresponding seawater sample itself (8.15) except for *U. fasciata*, *P. capillacea* and *C. sinuosa* (8.14, 8.12 and 8.14, respectively). The pH values of R1 and R2 were also lower than their corresponding seawater sample (8.13 and 8.12, respectively).

Dissolved oxygen (DO)

DO values of seawater for all the coated wood panels are slightly lower than the seawater sample itself (5.712 ml/l). The seawater medium contained wood coated panels with R1 and R2 showed the lowest DO concentrations compared to the other wood panels coated with different algae except for *P. capillacea* that showed the lowest seawater DO concentration (4.480 ml/l) (Table 3).

Nutrient salts

Ammonia (NH_3/N)

The wood panels' coated with R1 and R2 showed lower NH_3 concentrations (40.55-33.32 µmol/l) than their corresponding seawater sample itself (54.30 µmol/l). The NH_3 concentrations for the seawater medium containing wood coated panels with different types of algae showed also lower NH_3 concentrations than their corresponding seawater sample itself. Values fluctuated between maximum value of

41.90 µmol/l for *C. tomentosum* and minimum value of 22.85 µmol/l for *U. fasciata* (Table 4).

Table 3: The environmental impacts of the coated wood panels on some physicochemical parameters of the examined seawater samples after four weeks of immersion process

Source of biocide	Temperature ^{0}C	Salinity	pH	DO ml/l
Control	17.4	37.35	8.15	5.712
Corallina mediterranea	17.6	37.32	8.17	4.928
Codium tomentosum	17.7	37.31	8.15	5.376
Ulva lactuca	17.5	37.31	8.16	4.928
Ulva fasciata	17.5	37.32	8.14	5.376
Pterocladia capillacea	17.6	37.33	8.12	4.480
Jania rubens	17.7	37.33	8.15	5.040
Colpomenia sinuosa	17.6	37.32	8.14	5.176
R1	17.6	37.34	8.13	4.708
R2	17.6	37.34	8.12	4.816

Table 4: The environmental impacts of the coated wood panels on nutrient parameters (µmol/l) of the examined seawater samples after four weeks of immersion process

Source of biocide	PO_4/P	SiO_3/Si	NO_2/N	NO_3/N	NH_3/N
Control	0.86	1.45	0.50	9.06	54.30
Corallina mediterranea	0.43	4.35	4.20	4.41	23.25
Codium tomentosum	0.68	5.16	5.15	4.61	41.90
Ulva lactuca	0.65	3.65	2.98	5.23	30.75
Ulva fasciata	0.92	7.69	4.08	9.14	22.85
Pterocladia capillacea	0.54	4.57	4.40	4.75	39.30
Jania rubens	0.54	4.14	4.35	8.58	26.65
Colpomenia sinuosa	0.38	5.54	6.38	4.85	26.55
R1	0.43	5.11	6.57	2.80	40.55
R2	0.22	5.37	8.70	9.70	33.32

Nitrite (NO_2/N)

The NO_2^- concentrations of all seawater medium containing wood panels' coated are much higher than their corresponding seawater sample itself (0.50 µmol/l) and that of R1 and R2 showed the highest NO_2^- values of 6.57 and 8.70 µmol/l, respectively. The NO_2^- concentrations of seawater containing wood coated panels with different types of algae showed maximum value of 6.38 µmol/l and minimum value of 2.98 µmol/l corresponding to *C. sinuosa* and *U. lactuca*, respectively.

Nitrate (NO_3/N)

The NO_3^- concentrations for all seawater medium containing coated wood panels were lower than their corresponding seawater sample itself (9.06 µmol/l) except the coated wood panel by R2 (9.70 µmol/l) and the coated wood panel by *U. fasciata* (9.14 µmol/l).

Dissolved inorganic phosphate (PO_4/P)

PO_4^{3-} concentrations for all seawater medium contained coated wood panels were lower than their corresponding seawater sample itself (0.86 µmol/l) except for the seawater medium contained *U. fasciata* that recorded (0.92 µmol/l).

Silicate (SiO_3/Si)

The SiO_3^- concentrations for all seawater medium contained coated wood panels with different algae or reference biocides were higher than their corresponding seawater sample itself (1.45 µmol/l). The SiO_3^- concentration for seawater medium contained coated wood panels with *U. fasciata* showed the highest value (7.69 µmol/l), while the lowest SiO_3^- concentration value was for seawater medium contained coated wood panels with *U. lactuca* (3.65 µmol/l).

The physicochemical results showed that most seawater samples containing coated wood panels with different algal biocides showed slightly lower values in salinity, DO, PO_4^{3-}, NO_3^- and NH_3 concentrations than their corresponding seawater sample itself.

The effect of the contact media characteristics especially nutrient salts on the formation of the bacterial biofilm can be explained by the results of Stanley and Lazazzera (2004). They showed that nutrient availability regulates the depth of the biofilm in such a way that the maximal number of cells in a biofilm appears to occur at suboptimal nutrient concentrations. At either extreme, nutrient-rich or very nutrient-poor conditions, greater numbers of cells are in the planktonic phase where they have greater access to the local nutrients or can be distributed to a new environment. Similarly, quorum-sensing control of the formation of channels and pillar-like structures may ensure efficient nutrient delivery to cells in a biofilm. However, several factors seemed to be integrated together and led to the development of the adhered biofilm on these examined surfaces. Similarly, it was shown that among the conditions that affect biofilm development are temperature, pH, O_2 levels, hydrodynamics, osmolality, presence of specific ions, nutrients, and factors derived from the biotic environment. The integration of these influences ultimately determines the pattern of behavior of a given bacterium with respect to biofilm development (Goller and Romeo, 2008).

The microbiological examinations

Figs. (2-3) showed the bacterial plate count of the slime film on the tested wood panels after two and four weeks of seawater immersion, respectively.

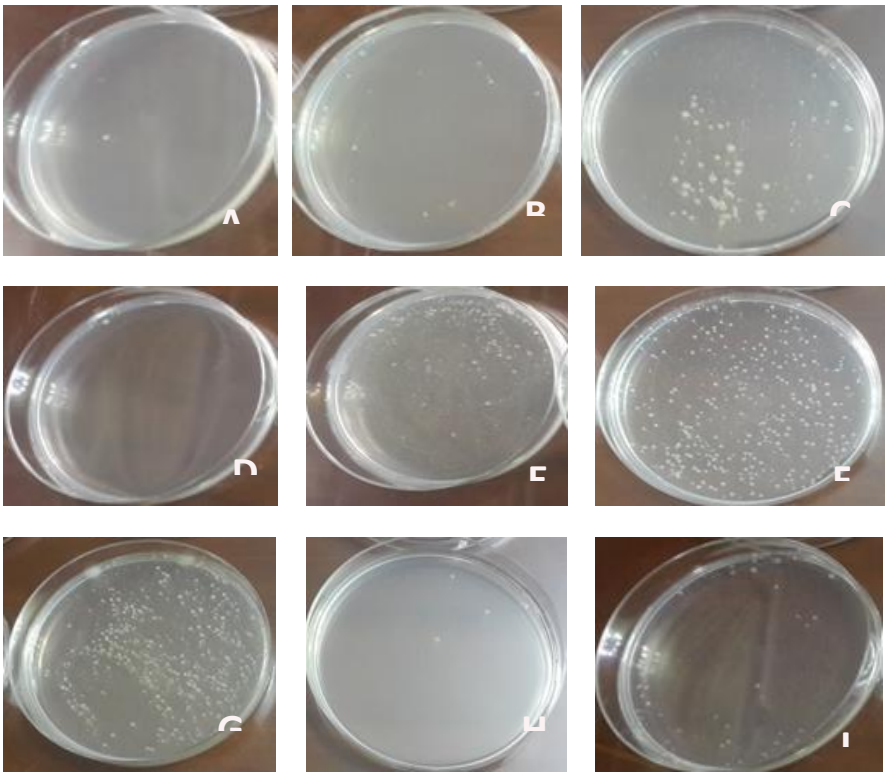


Fig. 2: Bacterial plate count for the slime film on the tested wood panels after two weeks of the immersion in the seawater. A: *Corallina mediterranea*, B: *Codium tomentosum*, C: *Ulva lactuca*, D: *Ulva fasciata*, E: *Pterocladia capillacea*, F: *Jania rubens*, G: *Colpomenia sinuosa*, H: Artificial paint (R1) and J: Artificial paint (R2)

Fig. 3: Bacterial plate count for the slime film on the tested wood panels after four weeks of the immersion in the seawater. A: *Corallina mediterranea*, B: *Codium tomentosum*, C: *Ulva lactuca*, D: *Ulva fasciata*, E: *Pterocladia capillacea*, F: *Jania rubens*, G: *Colpomenia sinuosa*, H: Artificial paint (R1) and J: Artificial paint (R2)

The bacterial count was presented in Fig. (4). The maximum values were 274 and 570 CFU/cm^2 for the binder without biocide, while the minimum values were for the most effective source of biocide, which was *Ulva fasciata* with the minimum bacterial count of 1 and 0 CFU/cm^2 after two and four weeks of immersion, respectively. However, after comparing the results it was noticed that all sources of biocides bacterial counts were lower than the blank itself.

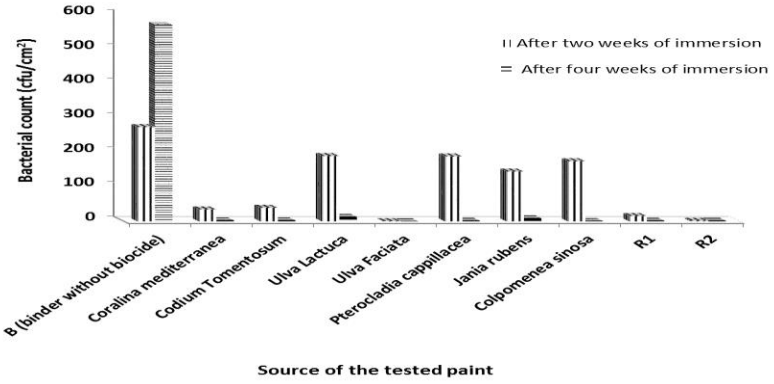


Fig. 4: Bacterial plate count for the slime film on the tested wood panels

The data presented in Fig. (5) showed only the suppressive effect % (+ve sign) after two and four weeks of seawater immersion. The maximum percentage was 100% while the minimum percentages were 30.7 and 97.7% after two and four weeks of immersion, respectively. The most effective source of biocide with the highest percentage of suppressive effect of 99.8 and 100% was *U. fasciata* after two and four

weeks of seawater immersion, respectively. It was noticed that the new biocides have a suppressive effect percentages better or equal to those of the artificial paints (R1 and R2) after two and four weeks of immersion in seawater.

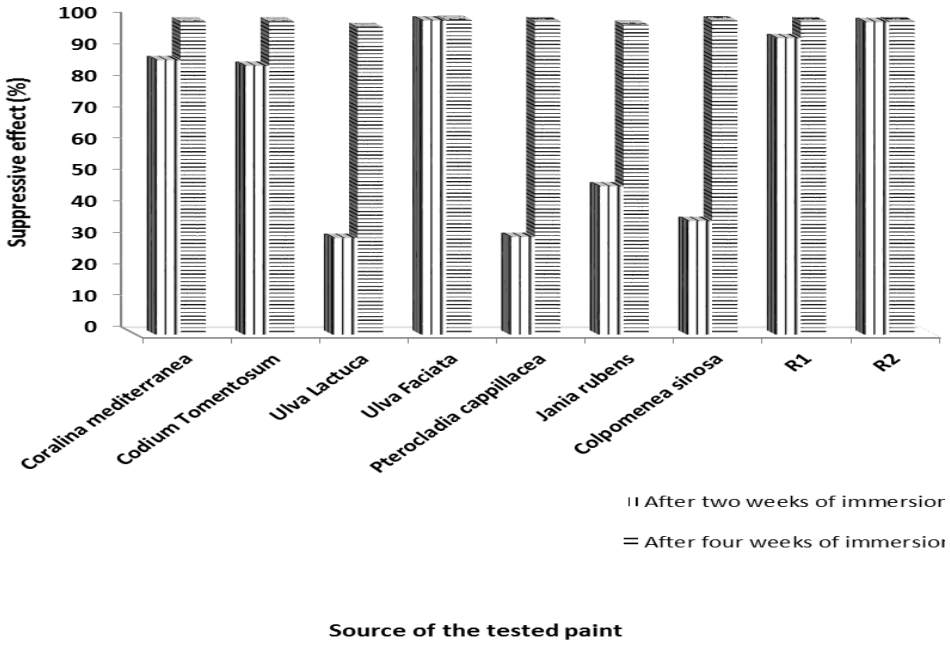


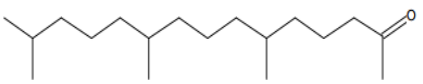
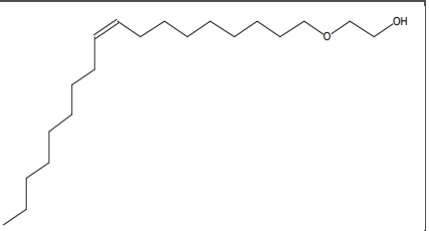
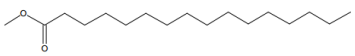
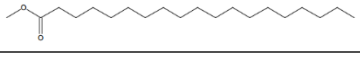
Fig. 5: Suppressive effect % of the slime film on the tested wood panels

The tested algal extracts can be arranged according to their antifouling activity as *U. fasciata*, *C. mediterranea*, *C. tomentosum*, *P. capillacea*, *C. sinuosa*, *J. rubens* and *U. lactuca*.

Characterization of the most potent algal extracts using GC-MS and FTIR

The GC-Ms was done for the most potent algal extracts which are: *C. mediterranea*, *C. tomentosu*m and *U. fasciata* (Table 5 and Figs. 6-8).

Table 5: The most potent algal extractions using GC-Ms

Compounds	Molecular formula	Molecular weight	Retention time (min)	Molecular structure
2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.277	19.60	
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.303	17.28	
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.256	21.06	
Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312.303	31.16	

The most compounds found in all algal extracts species are steroids and fatty acids. These mentioned algal extracts contain the most AF compounds. These

compounds can be categorized into ten types including fatty acids, lactones, terpenes, steroids, benzenoids, phenyl ethers, polyketides, alkaloids, nucleosides and peptides (Wang *et al.*, 2017). Most potent fatty acids present in all these three algal extracts are: hexadecanoic acid and nonadecanoic acid, while the most potent steroids are 2-pentadecanone, 6, 10, 14- trimethyl-, Ethanol, 2-(9-octadecenyloxy)-, (Z)-.

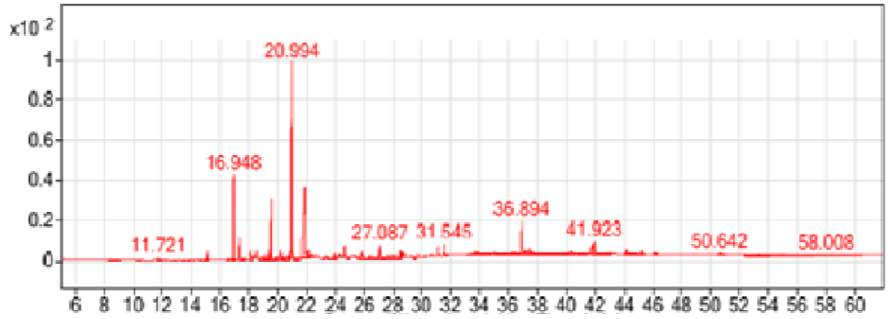


Fig. 6: GC-Ms of *C. mediterranea*

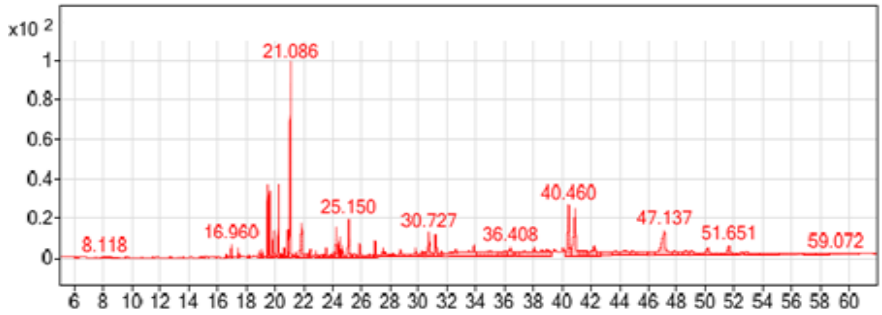


Fig. 7: GC-Ms of *C. tomentosum*

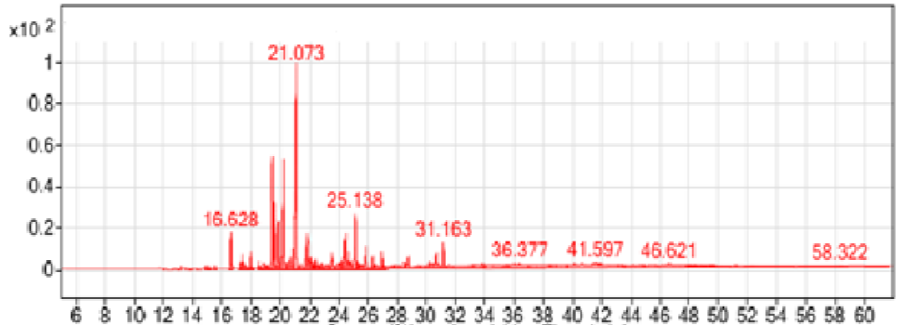


Fig. 8: GC-MS of *U. fasciata*

The major infrared absorption bands of the effective groups of *C. mediterranea*, *C. tomentosu*m and *U. fasciata* are presented in Table (6) and Fig. (9).

Table 6: Infrared absorption spectra of the most potent algal extract

Potent algae	γ_{N-H} &γ_{O-H}	$\gamma_{C=O}$	$\gamma_{C=O}$	γ_{C-N} &γ_{C-O}	γ_{C-O} &γ_{C-N}	γ_{C-H}	γ_{C-H}
Corallina mediterranea	3367.45	1634.79	1407.30	1196.30	1043.62	2921.27	872.17
Codium tomentosum	3372.50	1645.73	1457.76	1164.49	1098.54	2922.17	714.62
Ulva fasciata	3351.90	1721.23	1455.34	-	1093.77	2922.87	715.99

The absorption bands at 3367.45, 3372.50, 3351.90 cm^{-1} of the imino (N-H) and hydroxyl (O-H) groups are present in *C. mediterranea*, *C. tomentosu*m and *U. fasciata*, respectively. The carbonyl group band for all algae are present at 1634.79,

1645.73, 1721.23 cm^{-1}, respectively. The other stretching carbonyl group band for all algae are shown at 1407.30, 1457.76, 1455.34 cm^{-1}, respectively. The stretching C-N and C-O groups are present at 1196.30 and 1164.49 cm^{-1}, respectively for *C. mediterranea* and *C. tomentosu*m. The other stretching C-O and C-N groups are present at 1043.62, 1098.54, 1093.77 cm^{-1}, respectively for all algae. The two stretching C-H groups are present at 2921.27, 2922.17, 2922.87 and 872.17, 714.62, 715.99 cm^{-1}, respectively for all algae.

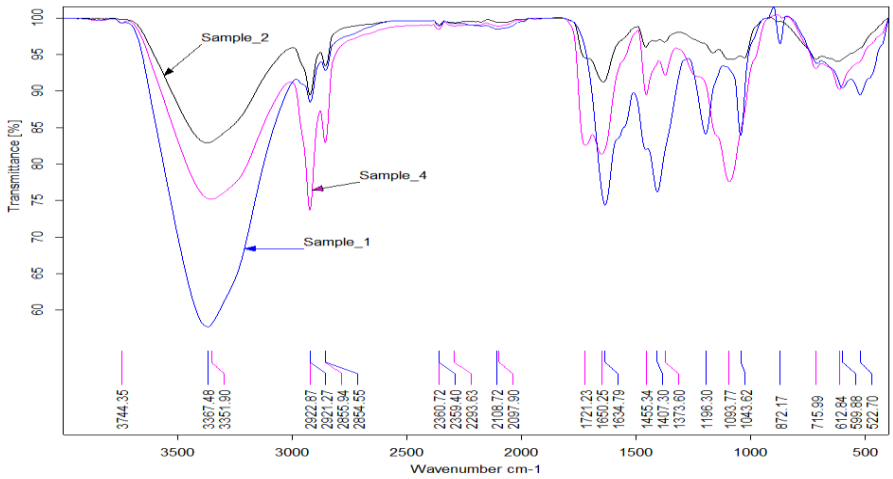


Fig. 9: Infrared spectra of the most potent algal extract. Sample 1: *C. mediterranea*, Sample 2: *C. tomentosu*m, Sample 4: *U. fasciata*.

The obtained results of both GC-MS and FTIR spectroscopy in this study indicated the extraction of fatty acids (hexadecanoic acid and nonadecanoic acid) and steroids (2- pentadecanone, 6, 10, 14- trimethyl- , Ethanol, 2-(9-octadecenyloxy)-, (Z)-) from *U. fasciata*, *C. mediterranea* and *C. tomentosum* which are recommended to be used as powerful antifoulant products.

Wladimir *et al.* (2016) suggested that *Laurencia translucida* cortical cells can produce fatty acid derivatives (docosane, hexadecanoic acid and cholesterol trimethylsilyl ether) and secrete them to the thallus surface, providing a unique and novel protective mechanism against microfouling colonization in red alga *L. translucida*. These secreted compounds can act as AF agents. These results were obtained using a laser scanning confocal microscopy (LSCM), HPLC coupled to a fluorescence detector and gas chromatography-mass spectrometry (GC-MS).

Bazes *et al.* (2009) indicated no cytotoxicity on the using of algal palmitic acid which is commonly found fatty acid in algae and it is promising in the development of environmentally friendly antifouling paints. Moreover, the pure compounds hexadecanoic acid, pentadecanoic acid, docosanoic acid, tetracosanoic acid, octadecanoic acid, eicosanoic acid and oleamide, isolated from *Sargassum granuliferum*, exhibited antifouling properties with broader activities (Bakar *et al.*, 2017).

Meanwhile, six terpenoids and two steroids have been isolated from three marine sponges as antifoulants, *viz*. manoalide, *seco*-manoalide, manoalide 25-acetate, (4E,6E)-dehydromanoalide from *Smenospongia* sp., (+)-curcuphenol and (+)-curcudiol from *Myrmekioderma dendyi*, and tri-2-amino-imidazolium halistanol sulfate and halistanol sulfate from *Topsentia* sp. These compounds showed antifouling activity but they were not toxic against cypris larvae of the barnacle *Balamis amphitrite* which is one of the most fouling agents (Tsukamoto *et al.*, 2009). In addition, other marine natural products were used in preparing two marine paints

based on tubeworms and *Sepia* shell. The two paints together with their blank (B) were applied on unprimed steel panel and immersed in seawater. The microbial examinations results indicated maximum inhibition of 43%, 25% and 64% for heterotrophic bacteria, actinomycetes and the sulfate reducing bacteria, respectively, on using *Sepia* shell based paint surfaces compared to the blank coated steel surfaces after 21 days of immersion (Tadros *et al.*, 2013; Tadros and Hermine, 2013).

The lipid content of green alga *U. lactuca* showed to be powerful in controlling the bacterial adhesion and other phytoplankton species (Tadros *et al.*, 2006). Where the use of the algae free lipids in the paint films resulted in reducing the microbial communities and different phytoplanktonic species in the surrounding marine environment compared to the binder coated panels (Tadros and El-Naggar, 2007). Also another study aimed to estimate the suppressive effect of some coatings containing *U. lactuca* free lipid on the well-defined marine isolate *Staphylococcus aureus* ATCC 6538 as a model for the slime film forming bacteria. In addition, the effect of the leached components from these coated surfaces containing algae free lipid on the characteristics of the contact seawater medium was investigated. The suppressive effects showed that the use of 5% algae free lipid in the dry paint film lead to highest suppressive effect on the *Staph. aureus* cells in both the surrounding medium and on the tested coated panels (Tadros *et al*., 2009).

CONCLUSION

These studies confirmed the ability of using such alternative marine natural products extracted from different marine algae as antifouling agents. The fatty acid and steroids extracted from different seaweeds are very promising as safe, economic additives for antifouling paint manufacture. The obtained steroids, also in this study indicated non harmful effect for the aquatic living organisms, have been applied.

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ARABIC SUMMARY

انتاج الدهانات المضادة للحشف البحرى باستخدام مستخلصات الطحالب الآمنة بيئياً وتطبيقها معملياً

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يتكون الحشف نتيجة لتراكم النمو البكتيرى والطحالب واللافقاريات على كلا من الأسطح الطبيعية أو الصناعيةً الموجودة في البيئة المائية. ويجب أن تخضع السفن لعمليات التنظيف والصيانة الدورية وذلك لمقاومة الحشف المتكون على سطحها. ويهدف هذا العمل الى تقييم مضادات للحشف جديدة ناتجة من الطحالب البحرية، مع دراسة تأثيرها المثبط لتكون الفيلم البكتيري علي تلك الاسطح. بالإضافة الى انه تم دراسة تأثير تلك المكونات الطحلبية والتي تم استخدامها لطلاء الأسطح الخشبية تحت الاختبار كمثال للسفن الخشبية المتداولة في البيئة البحرية على خصائص ماء البحر الملامس لهذه الألواح الخشبية، كما تم مقارنة ذلك مع نوعين من الطلاءات البحرية والمستخدمه تجاريا كالمضادات للحشف. ولتقييم التأثير المثبط للطلاءات المحتوية على الطحالب تم استخلاص وخلط المكونات (مادة الربط ، الأصباغ ، المثبت، المذيب) بنسب مئوية مختلفة . وكانت الدهانات التى تم الحصول عليها جيدة الخلط ويمكن استخدامها لفترات طويلة بدون تشققات قبل دمجها مع الطحالب المختلفة. تم تطبيق تركيبات الطلاءات المختلفة على الألواح الخشبية ثم تم غمرهم فى كئوس زجاجية معقمة مملوءة بماء البحر تم جمعها من الميناء الشرقي بالإسكندرية، مصر. حيث تم الفحص الميكروبيولوجي للألواح بعد أسبوعين وأربعة أسابيع من الغمر كما تم قياس المعاملات الفيزيوكيميائية المختلفة (درجة حرارة، درجة ملوحة، الأس الهيدروجيني، الأكسجين الذائب، الأملاح المغذية) لعينات مياه البحر حول الألواح المختبرة بعد أربعة أسابيع من الغمر. وقد وجد أن استخدام الطحالب فى الطلاء الجاف أدى الى أعلى نسبة مئوية للتأثير المثبط لتكون الفيلم البكتيري علي الاسطح تحت الاختبار كما اتضح أن استخدام كلا من مستخلصات : الأولفا فشياتا، كورالينا ميديتيرانيا وكوديوم تومنتوسم على الألواح الخشبية المختبرة كان لها أعلى تأثير مثبط ويمثل 100 و 99.6 و 99.5 % على التوالي. تطابقت تلك النتائج مع توصيف GC-MS (كروماتوغرافيا الغاز-مقياس الطيف الكتلي) وFTIR (مطياف الأشعة تحت الحمراء). والتي اكدت استخلاص مركبات تنتمي الي الاحماض الدهنية والاستيرويدات والتي ثبت فعالياتها كمضادات للبكتيريا بانواعها.