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Antifouling evaluation of extracts from Red Sea soft corals against primary biofilm and biofouling

Yosry Abdel Aziz Soliman[1], Ahmed Mohammed Brahim[1*], Ahmed Hussein Moustafa[2], Mohamed Abdel Fattah Hamed[1]

[1]*Marine Chemistry Lab., Marine Environment Division, National Institute of Oceanography and Fisheries, Suez, Egypt*

[2]*Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt*

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ABSTRACT

Objectives: To evaluate antifouling property of extracts from Red Sea soft corals against primary biofilm and biofouling.

Methods: Seven species of soft corals *Sarcophyton glaucum* (a), *Sinularia compressa*, *Sinularia cruciata* (a), *Heteroxenia fuscescens* (a), *Sarcophyton glaucum* (b), *Heteroxenia fuscescens* (b) and *Sinularia cruciata* (b) were chosen to test their extracts as antibacterial and antifouling agents in Eastern Harbour of Alexandria, Mediterranean Sea. Bioactive compounds of soft corals were extracted by using methanol and concentrated under vacuum. The residues of extracts were mixed in formulation of inert paint which consisted of rosin, chlorinated rubber and ferrous oxide against micro and macro fouling organisms. The formulated paints were then applied on PVC panels twice by brush, hanged in a steel frame and immersed in Eastern Harbour of Alexandria Mediterranean Sea followed by visual inspection and photographic recordings.

Results: After 185 days of immersion in seawater, the antifouling results agreed with the antibacterial results where extracts of *Sinularia compressa* and *Heteroxenia fuscescens* (b) gave the best activity against marine fouling tubeworms and barnacles. The inhibition activity was correlated with the major functional groups (hydroxyl, amino, carbonyl, aliphatic (fatty acids), C═C of alkene or aromatic rings and C−Cl of aryl halides) of the extracts.

Conclusions: The strong antifouling activity makes them promising candidates for new antifouling additives. After the screening and application of natural organic compounds from soft corals, marine organisms show activity against micro and macro fouling organisms.

1. Introduction

Marine bio-fouling can be defined as the growth of unwanted organisms on the surface of artificial structures immersed in water [1,2]. Bio-fouling causes huge material and economic costs of maintenance of marine structures, naval vessels, and seawater pipelines [1]. It is estimated that governments and industry spend over $6.5 billion annually to prevent and control marine bio-fouling [3]. Further, ecological implications of bio-fouling include increased carbon emission and potential dispersion of invasive alien species [4–6]. Antifouling is the process of controlling or mitigating the settlement of fouling organisms on a surface. Commercial antifouling techniques include mechanical cleaning, biocides, toxic antifouling coatings and foul release or easy clean coatings. Amongst the above, antifouling paints containing toxic chemicals are the main strategies used against bio-fouling in the past. Tri-butyl tin was the most effective component in antifouling paints which was detrimental, not readily degraded in the natural environments and had non-targeted toxicity on organisms [7]. This property has led the International Maritime Organization to prohibit its application to ships since 17 September 2008 [8]. The substitutes of tributyl tin, such as Irgarol 1051 and

*Corresponding author: Ahmed Mohammed Brahim, Marine Chemistry Lab., Marine Environment Division, National Institute of Oceanography and Fisheries, Suez, Egypt.

E-mails: ahmed_pending@yahoo.com, ah_hu_mostafa@yahoo.com (A.M. Brahim).

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Diuron, have also been found to be harmful to many non-targeted organisms [7,9]. Hence, alternative and environmentally acceptable, safe and effective antifouling substances are needed for incorporation into antifouling coatings, and these may include natural products isolated from certain marine organisms [10]. Incorporation of natural repellent products into antifouling paints has been tried by some researchers [11,12]. For this, a wide range of marine natural products have been screened for their activity concerning antimicrobial, antifungal, antialgal and antilarval properties [10,13,14]. Compounds with antifouling potential have been studied intensively in various marine sponges [15,16] and algae [17–19]. Marine natural products or crude extracts with antifouling activity have been reported from many marine organisms including marine bacteria, seaweeds, sea grasses, bryozoans, ascidians, cnidarians and sponges [10,20,21]. Antifouling and biological activities of marine macrophytes have been extensively studied by many researchers in various species of mangroves [6] seaweeds [22] and sea grasses [23,24]. In continuation to the previous study, this work depended on the extraction of natural products of soft corals [25] from Hurghada and Sharm El Sheikh to evaluate the bioactive and antifouling. The active functional group of the extracted organic compounds against biofouling was detected by using infrared spectroscopy.

2. Materials and methods

2.1. Sample collection, identification and extract preparation

The soft corals were collected by using SCUBA diving at different depths from Hurghada and Sharm El Sheikh, Egypt, on the Red Sea, in November 2013 and April 2014, respectively. Then, the collected samples were kept at −20 °C at the National Institute of Oceanography and Fisheries, Suez branch. Red Sea soft corals from Hurghada were identified as *Sarcophyton glaucum* (*S. glaucum*) (a) 1.5 m, *Sinularia compressa* (*S. compressa*) 2 m, *Sinularia cruciata* (*S. cruciata*) (a) 1.5 m and *Heteroxenia fuscescens* (*H. fuscescens*) (a) 2 m and the corals from Sharm El Sheikh as *S. glaucum* (b), 0.5 m, *H. fuscescens* (b), 10 m and *S. cruciata* (b), 10 m. The extraction processes of the bioactive organic compounds were as follows: About 1 200 g of *S. glaucum* (a), 411.4 g of *S. compressa*, 713.77 g of *S. cruciata* (a), 329.27 g of *H. fuscescens* (a), 408.907 g of *S. glaucum* (b), 413.762 g of *H. fuscescens* (b) and 284.683 g of *S. cruciata* (b) were prepared. After cleaning and cutting into small pieces, methyl alcohol was used to extract the bioactive compounds three times for 10 d. The extract was concentrated under vacuum, the residue was washed three times by using ethanol to eliminate the inorganic salts, and then the filtrate was evaporated under vacuum to afford the bioactive organic compounds as crude.

2.2. Bacterial characterization

A microtitre assay by Andrews [26] was performed to determine the minimal inhibitory concentration (MIC) of the crude extract on different bacterial isolates. All extracts were diluted with dimethyl sulfoxide to prepare stock solutions of 100 mg/mL. A serial dilution of each stock solution was then performed into sterile nutrient broth. Different concentrations from each pure extract were made (100, 50, 25, 10, 5, 1, 0.5, and 0.1 mg/mL). For each concentration in nutrient broth 75 μL was pipetted into horizontal wells of well cell culture plate (1 well per concentration per bacterial isolate). The above procedure was repeated for each extract and the combinations, giving final well concentrations. Each plate was incubated in incubator at 32 °C for 24 h. Following incubation wells were observed for turbidity. MICs were taken as the lowest concentrations not showing any visible growth. Minimum bactericidal concentration (MBC) was also determined by removing 2 μL volume of the medium from each microtitre plate well and spotting onto sensitive agar. Agar plates were incubated for 18 h at 30 °C. Any growth observed from the spots was designated as an ineffective bactericidal concentration of extracts [27].

The well-cut diffusion technique was used to test the ability of different concentrations from the pure extract to inhibit the growth of indicator bacteria. About 50 mm of seawater agar medium inoculated with indicator microorganism was pored after solidification into plates. Wells were punched out by using 0.5 cm cork borer, and each of their bottoms was then sealed with two drops of sterile water agar. One hundred micro-liters of tested extracts were transferred into each well. All plates were incubated at 30 °C for 24 h; the detection of clear inhibition zone around the wells was an indication of antimicrobial activities of the different isolates.

2.3. Paint preparation, panel and frame preparation and field anti-macrofouling assays

The extracts were incorporated into inert matrix ingredients which consisted of 40 g rosin, 20 g chlorinated rubber, 10 g ferrous oxide, 20 mL dioctyl phthalate and 40 mL xylene in porcelain bottle jar (1 L) containing porcelain balls for stirring the components to form homogeneous paint. The formulated paints applied on PVC panels by brush were immersed in seawater of Eastern Harbour of Alexandria to investigate their antifouling profile under harsh marine conditions such as microorganisms in hydrothermal vents, corals …*etc*. Seven coating paint formulations (AF_1, AF_2, AF_3, AF_4, AF_5, AF_6 and AF_7) have been prepared by incorporating the extracts of *S. glaucum* (a), *S. compressa, S. cruciata* (a), *H. fuscescens* (a), *S. glaucum* (b), *H. fuscescens* (b) and *S. cruciata* (b) (2 g of tested extract/48 g of paint), respectively. In addition, the inert paint formulation was used as a control.

A 0.2 cm thick sheet of PVC panel was cut into 10 cm × 15 cm × 0.2 cm panels which were roughened by using emery papers at different grades from a coarse one to finer one. These panels were coated from both sides with two successive coats of the formulated paint. The paints were prepared by blending definite amounts of binder, pigments, plasticizer, then extracted compounds and solvents in a high-speed centrifuging ball mill, and were allowed to dry for 2 d between each coating. The coated panels were connected to the testing iron frames with nylon threads through nails bored in the panels.

All panels were immersed in the Eastern Harbour of Alexandria at a depth of 1.5 m, where the antifouling performance of each coated panel was studied periodically from 5 May 2015 to 10 November 2015 by visual inspection and photographic recordings. After a definite time, the panels were taken out of the sea, carefully washed with seawater and photographed. Then, they were immediately placed into the seawater to continue the

test. The coverage percents of marine fouling organisms over different time intervals were used as parameters to express macrofouling propensity.

2.4. Seawater sampling and analytical procedure

Surface seawater samples were collected from Alexandria Eastern Harbour using Niskin reversing bottle. Seawater temperature was measured by using an inductive portable thermometer. Salinity was measured by using Bench/portable conductivity meter. The pH-value of water samples was measured to about 0.1 unit in situ by using a portable pH-meter (Orion Research model 210 digital pH-meter) after necessary precautions in sampling and standardization processes. Dissolved oxygen was determined according to the classical Winkler's method modified by Grasshoff [28]. Oxidizable organic matter concentrations were determined by permanganate oxidation method [29]. Nutrients salts, nitrite, nitrate, ammonia, silicate and phosphate were measured according to Grasshoff by using a single beam spectrophotometer model Beckman Du-6 visible-UV, and sulfate was measured by using barium method which was mentioned in the American Public Health Association standard method [30].

2.5. Fourier transform infrared spectroscopy (FTIR) and gas chromatography/mass spectrometry (GC–MS) analysis

The IR spectra (KBr disc) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer for each extract and the methanol extract of the soft coral *S. compressa* was analyzed by GC–MS (Make: Fisons GC8000 series and MS: md800) [31].

3. Results

3.1. Bioassay of soft coral extracts

As parameters of antibacterial efficacy, the MIC/MBC of most potent soft coral extracts were estimated against tested marine bacteria by using the micro-dilution broth susceptibility test. The obtained results were summarized as follows: MBC varied in several orders of magnitudes from the MIC, with maximum synergistic bactericidal and bacteriostatic action being achieved for extract of *S. compressa* against *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC6538 was (MIC/MBC = 1/5 mg/mL), against *Staphylococcus aureus* (*S. aureus*) ATCC6538 was (MIC/MBC = 0.5/10 mg/mL), against *Escherichia coli* (*E. coli*) was (MIC/MBC = 0.5/1 mg/mL) and against *P. aeruginosa* ATCC6739 was (MIC/MBC = 25/50 mg/mL). Extracts of *S. glaucum* (a) and *S. cruciata* (a) showed good activity against *P. aeruginosa* ATCC6538 was (MIC/MBC = 50/100 and 5/100 mg/mL), against *S. aureus* ATCC6538 was (MIC/MBC = 5/10 and 25/50 mg/mL) and against *E. coli* was the same (MIC/MBC = 25/50 mg/mL), respectively. But extract of *H. fuscescens* (a) showed no activity in any of the concentrations studied.

Methanol extracts of Red Sea soft corals [*S. glaucum* (a), *S. compressa*, *S. cruciata* (a) and *H. fuscescens* (a)] were evaluated for their antibacterial activity. In the antibacterial assay, they

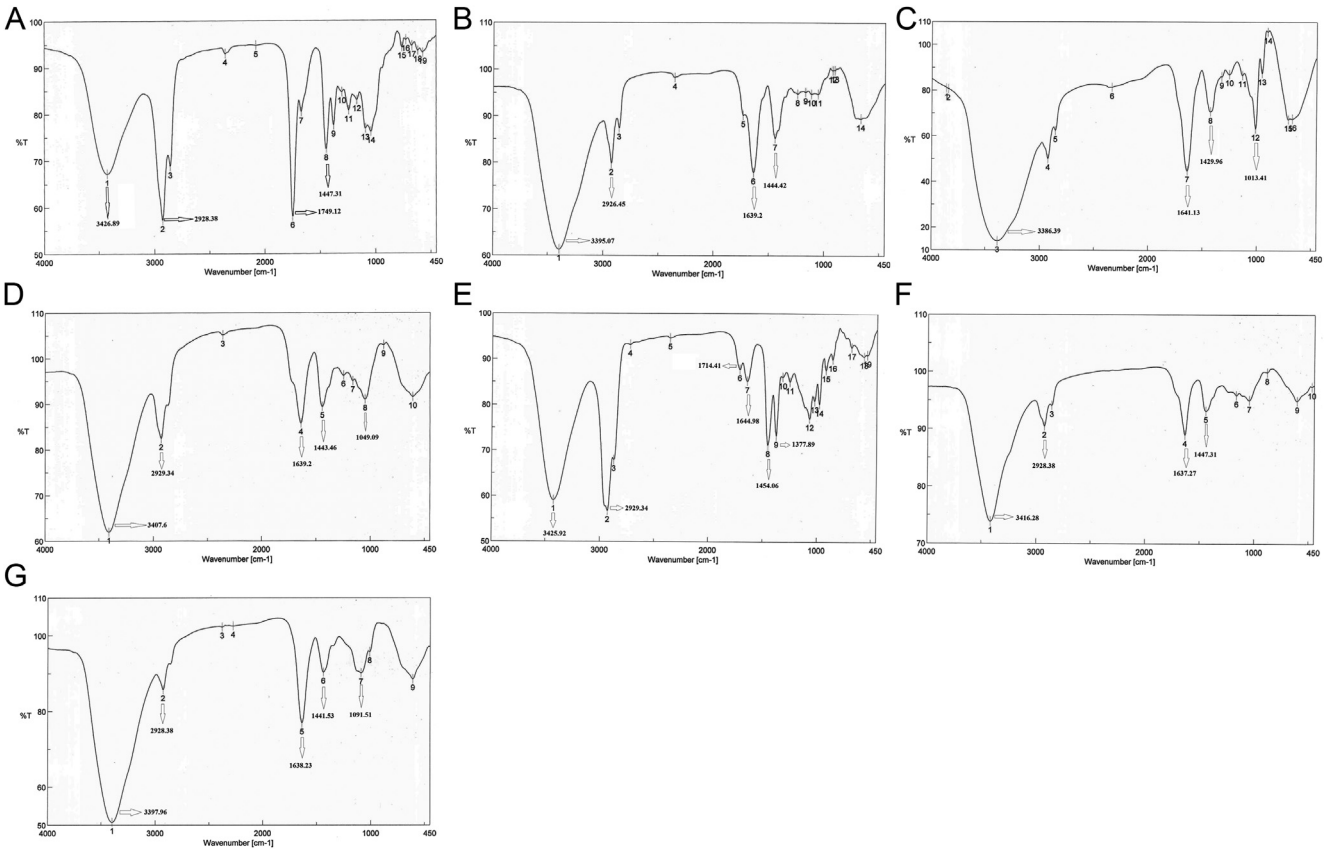

Figure 1. IR spectra of soft coral extracts.
A: *S. glaucum* (a) extract; B: *S. compressa* extract; C: *S. cruciata* (a) extract; D: *H. fuscescens* (a) extract; E: *S. glaucum* (b) extract; F: *H. fuscescens* (b) extract; G: *S. cruciata* (b) extract.

displayed inhibition zones of 25, 40, 20 and 0 mm against *S. aureus*. Furthermore, their respective inhibition zones against *P. aeruginosa* were 20, 30, 18 and 0 mm. Also, they showed inhibition zones of 20, 25, 16 and 0 mm against *E. coli.*

3.2. Antifouling tests of soft coral extracts

The visual inspection of antifouling paints for each extract was taken after 7, 13, 20, 39, 69, 116 and 185 days of immersion. After 7 days of immersion, the slime film which consisted of marine bacteria, diatoms and micro algae was absent on control (paint formulation without extract), AF_1, AF_2, AF_3, AF_4, AF_5, AF_6 and AF_7 panels except uncoated panel. After 13 days of immersion, The uncoated panel was covered with about 60% of mucous slime film (marine bacteria and green algae) and 10% small tubeworms while the control, AF_1, AF_2, AF_3, AF_4, AF_5 and AF_7 panels contained slime film on the edges of the panels. Control, AF_1, AF_3, AF_4, AF_5 and AF_7 panels contained small tubeworms with 5%, 1%, 2%, 2%, 1% and 2% respectively concentrated in the center of the surfaces. After 20 days of immersion, the uncoated panel was nearly covered with slime film while tubeworms and brown algae appeared on the surface of the control panel with 15% and 5%, respectively. But on the AF_1 panel, the percent of tubeworms reached about 10% and 2% brown algae also appeared while the barnacles still were absent on all panels. But 2% tubeworms appeared on the edges of AF_2 panel. AF_3 panel still contained the same amount of tubeworms as mentioned before. AF_4 panel contained tubeworms with 10%. AF_5 and AF_6 panels contained small tubeworms with the same percent 1% and green layer spread over the surface of the panels but AF_5 contained only brown algae with 1%. This may be due to the release of these extracts which existed in the paint as a biocide. AF_7 panel contained 30% tubeworms, 12% green layer spread over the surface of the panel and 5% brown algae. After 39 days of immersion, the formation of slime film facilitated the growth of tubeworms on the surface of uncoated panel. The percent of tubeworms decreased to reach 3% but barnacles started to appear on the surface of control panel. AF_5 and AF_6 panels contained green algae with 15% and 5%, respectively on the edges of these panels. Brown algae appeared with 15% and 1% on the lower part of the control and AF_5 panel respectively. AF_7 panel was completely fouled with brown algae, tubeworms and barnacles. These results were also accompanied by higher temperature value than that in the previous days, beside the lowest pH and nitrate values. After 69 days of immersion, the uncoated panel was completely covered with about 10% green algae, 40% red algae, 40% tubeworms and 10% barnacles. The percent of red algae was 5% appeared on the edges of the control panel and 2% barnacles also appeared on the lower part of the same panel. The percent of barnacles which formed on the edges

Table 1
Identification of functional groups through FTIR analysis.

Name of species	Frequency (cm^{-1})	Bond	Functional group
S. glaucum (a)	3426 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines
	2928 and 2858 (m,n)	C−H stretch	Alkanes
	2079 (w,n)	C≡N stretch	Nitriles
	1749 (s,sh)	C=O stretch	Esters
	1671 (s,sh)	C=O stretch	Amides
	1170 (m,sh)	C−O stretch	Alcohols, esters and ethers
	1447 (m,sh)	C=C stretch	Alkenes
S. compressa	3395 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines
	2926 and 2857 (s,n)	C−H stretch	Alkanes
	1732 (s,sh)	C=O stretch	Esters
	1639 (s,sh)	C=C stretch	Alkenes
	1171 (w,n)	C−O stretch	Esters and ethers
	905 (w,n)	C−Cl stretch	Aryl halides
S. cruciata (a)	3386 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines
	2926 and 2858 (s,m)	C−H stretch	Alkanes
	1641 (s,sh)	C=C stretch	Alkenes
	1137 (w,n)	C−O stretch	Esters and ethers
H. fuscescens (a)	3407 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines
	2929 (s,sh)	C−H stretch	Alkanes
	1639 (m,n)	N−H bending	Amines
	879 (w,n)	C−Cl stretch	Aryl-halides
S. glaucum (b)	3425 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines (H-bonded)
	2929, 2873 (s,sh)	C−H stretch	Alkanes
	1714 (w,n)	C=O stretch	Ketones
	1644 (m,n), 1454 (m,sh)	C=C stretch	Alkenes or aromatic ring
	1070 (w,n)	−O− stretch	Alcohols, carboxylic acids, esters and ethers
	859, 683 (w,n)	di- and multi-substitution	Aromatic ring
H. fuscescens (b)	3416 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines (H-bonded)
	2928 (m,n), 2861 (w,n)	C−H stretch	Alkanes
	1637 (m,n)	C=N stretch	Azines or azole ring
	1447 (m,n)	C=C stretch	Alkenes or aromatic ring
	885, 607 (w)	mono substituted	Aromatic ring
S. cruciata (b)	3397 (s,b)	O−H and N−H stretch	Alcohols, phenols and amines (H-bonded)
	2928 (m,w)	C−H stretch	Alkanes
	2278 (w)	C≡N stretch	Nitriles
	1638 (s,sh), 1441 (m,n)	C=C stretch	Alkenes or aromatic ring
	1091 (w,b)	C−O stretch	Alcohols, esters and ethers

m = medium, w = weak, s = strong, n = narrow, b = broad, sh = sharp.

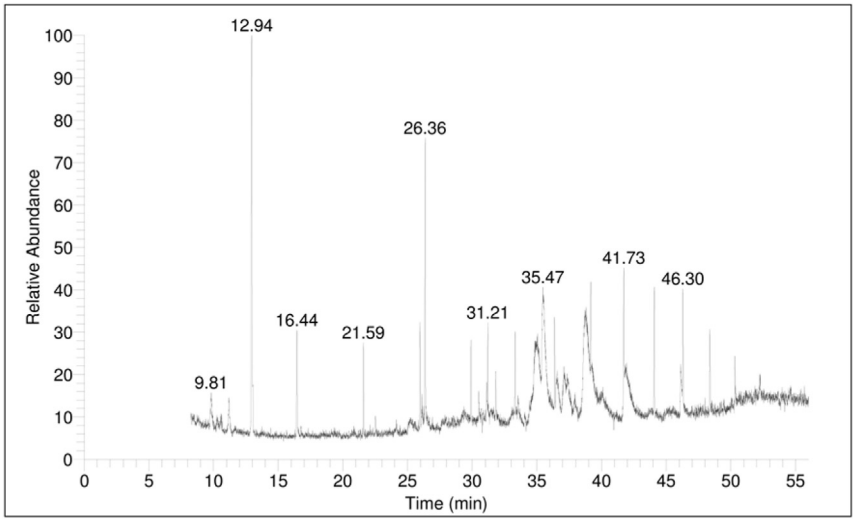

Figure 2. Gas chromatogram of *S. compressa* extract.

of AF_1 panel was 2%. The green algae layer which formed before decreased from AF_2 panel this time. About 10% tubeworms and 1% barnacles spread on the surface of AF_3 panel while AF_4 panel contained tubeworms spread on the surface with 10%, barnacles with 1% and red algae with 2% appeared on the edges of the same panel. The formation of green algae, red algae and barnacles on the surface of AF_5 panel were observed with 5%, 5% and 10%, respectively. Also, the AF_5 extract was still resistant to the formation of tubeworms. The formation of green algae with 5% and red algae with 6% on the edges and middle of AF_6 panel was observed. Much of brown algae, tubeworms and barnacles which formed before on AF_7 panel came off this time due to the wind existed in this area. AF_7 panel contained few barnacles with 20% and brown algae with 5% due to the effect of extract releasing from AF_7 paint formulation. The disappearance of tubeworms from the surface of AF_7 panel was noticed. These results were accompanied by the highest temperature, lowest dissolved oxygen and silicate values. After 116 days of immersion, the uncoated and control panels were completely fouled with heavily tubeworms and barnacles except the lower part of control panel. Low amounts of tubeworms and barnacles were formed on AF_1 panel due to the continuous release of the extract. The surfaces of AF_2 and AF_3 panels contained tubeworms and barnacles with the same percent 65% and 20%, respectively. While the amount of the red algae which formed before on AF_2 panel decreased due to the release of extract and adhesion weakness between the red algae and the surface of paint formulation. The surface of AF_4 panel contained 45% tubeworms and 15% barnacles. The surface of AF_5 contained 40% tubeworms and 30% barnacles. The surface of AF_6

Table 2
Mass spectrometry of possible compounds existed in *S. compressa* extract (m/e).

Name of compounds	Calculated	Expected	Fragments
1,7,7-trimethyltricyclo[2.2.1.$0^{2,6}$]heptanes 2	136.13	136.0 (15%)	121, 93 (base peak), 91, 41 and 43
3,4,5,6-tetrakis(4-chlorophenoxy)phthalonitrile 3	631.99	640.0 (4%) (M^{+2})	636, 635, 634 (base peak), 633, 632
6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane 4	136.13	137.0 (5%) (M^{+1})	93 (base peak), 91, 79, 69, 41
(1*s*,4*s*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane 5	154.14	154.0 (50%)	139, 108, 81 (base peak), 71, 64 and 43
(1*r*,4*s*)-4,7,7-trimethylbicyclo[2.2.1]heptan-2-one 6	152.12	152.0 (50%)	159, 81, 69 (base peak)
1,3-bis(4-chlorobenzyl)-5,6-dihydrobenzo[*f*]quinazoline 7	430.10	434.0 (8%)	432, 430, 429 (base peak)
2,6-di-*tert*-butyl-4-methylphenol 8	220.18	220.0 (15%)	205 (base peak) and 57
Methyl hexadecadienoate 9	266.22	266.0 (12%)	96, 95, 81, 67 (base peak), 55 and 41
Methyl 14-methylpentadecanoate 10	270.26	270.0 (11%)	87 and 74 (base peak)
3,3,3-trifluoro-2-hydroxy-1-phenylpropan-1-one 11	204.04	204.0 (5%)	107, 105 (base peak) and 77
Meso-tetraphenyl-2,3-cis-dihydroxy-2,3-chlorin 12	648.25	648.0 (2%)	632, 631, 630 (base peak) and 614
(*Z*)-1-methyl-4-(6-methylhepta-2,5-dien-2-yl)-7-oxabicyclo[4.1.0]heptane 13	220.18	220.0 (2%)	109, 93, 67, 57, 55 and 43 (base peak)

panel contained 20% tubeworms on the edges of panel. The surface of AF_7 panel contained 25% barnacles and 10% tubeworms. Brown algae disappeared, which may be due to the extract releasing of soft coral *S. cruciata* (b) sp existed in AF_7 paint formulation. These results were accompanied by highest oxidizable organic matter and silicate concentrations, beside lowest nitrite concentration. After 185 days of immersion, the uncoated control, AF_2, AF_3, AF_4, AF_5, AF_6 and AF_7 were completely fouled with barnacles, tubeworms and red algae while the AF_1 panel contained fouling with 90%. This may be due to complete releasing of the extracts from the marine paint formulation. These results were accompanied by the lowest temperature, salinity and nitrate values as well as highest dissolved oxygen and sulfate concentrations.

3.3. FTIR analysis

The FTIR analysis of the soft coral extracts of *S. glaucum* (a), *S. compressa*, *S. cruciata* (a), *H. fuscescens* (a), *S. glaucum* (b), *H. fuscescens* (b) and *S. cruciata* (b) was done. Figure 1a–g demonstrated the presence of the principle functional groups as NH, OH, C═O, C═N, C−H aliphatic, C−O−C and C═C which were shown in the Table 1.

3.4. GC–MS analysis of S. compressa extract

GC–MS analysis of *S. compressa* extract gave the chromatogram (Figure 2). Mass spectrometry showed the m/e (parent ion) for some compounds; tricyclene 2 (136, 15%), 3,4,5,6-tetrakis(4-chlorophenoxy)phthalonitrile (3) (640, 4%), α-pinene 4 (137, 5%) and the mass spectrometry for the other compounds was written in the Table 2 and the structure suggestion of each compound.

4. Discussion

Badria investigated bioactivity-guided fractionation of an alcohol extract of the soft coral *Sarcophyton* sp. collected from coral reefs near Hurghada, Red Sea, Egypt which afforded a new lactone cembrane diterpene, sarcophytolide [32].

Ali studied the antifouling activity of crude extracts of 5 common Red Sea soft corals [33]. The extracts mixed with a marine paint were applied on PVC panels immersed in the seawater of Suez Bay (Red Sea). Extracts of *Sinularia heterospiculata* and *Sinularia variabilis* showed the highest and potent wide spectrum antifouling activity, particularly in the first 17 d of fouling formation. Extracts of *Sinularia polydactyla* exhibited significant selective inhibition against settlement of barnacles, while the extracts of *Lithophyton arboreum* showed significant antifouling activity against the latter successional stages of tubeworms [33].

According to the obtained results, extract of *S. compressa* gave the best MIC and MBC against the three bacterial pathogens while extract of *S. glaucum* (a) and *S. cruciata* (a) showed good MIC and MBC against *S. aureus* and *E. coli*. But extract of *H. fuscenscens* (a) showed no MIC and MBC at any studied concentration. Extract of *S. compressa* gave the best antibacterial activity against the three bacterial pathogens while extracts of *S. glaucum* (a) and *S. cruciata* (a) had good antibacterial activity against the three bacterial pathogens. But extract of *H. fuscenscens* (a) displayed no antibacterial activity against the three bacterial pathogens. So the extracts of *S. compressa*, *S. glaucum* (a) and *S. cruciata* (a) showed higher inhibiting activity against primary biofilm forming bacteria than the other extracts. Green algae spread over the surface of AF_1, AF_2, AF_3 and AF_4 panels and the tubeworms which were noticed before disappeared after 39 days of immersion and this may be due to the release of these extracts. They appeared to weaken the bio adhesive bond between the tubeworms and the surface of paint formulation so the tubeworms were fallen in the sea due to the current and wind in the Eastern Harbour area. The disappearance of tubeworms from AF_5 and AF_6 panels was also noticed which may be due to the release of extracts of *S. glaucum* (b) and *H. fuscescens* (b). After 69 days of immersion, extract of *S. glaucum* (a) showed the limitation of growth of green algae. Extract of *S. compressa* also appeared resistance to the formation of tubeworms. Extracts of *S. glaucum* (b) and *H. fuscescens* (b) limited the growth of barnacles and tubeworms. After 116 days of immersion, extract of *S. glaucum* (a) prevented the growth of tubeworms and barnacles while extracts of *S. compressa* and *H. fuscescens* limited the growth of red algae. Extract of *H. fuscescens* (b) restricted the growth of green, red algae and barnacles. Hence, extracts of *S. compressa*, *H. fuscescens* (b) and *S. glaucum* (a) displayed the best limitation of growth of tubeworms and barnacles through the period of the experiment due to releasing of the extracts from the marine paint formulation into seawater. This limitation is correlated with the presence of functional groups (hydroxyls, amino, carbonyl of ester and amide and double bond for alkenes and aromatic ring) according to the results of IR and GC–MS analyses well as structure suggestions of compounds presented in *S. compressa* extract.

Conflict of interest statement

We declare that there is no conflict of interests.

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