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Exploring the antifouling properties of compounds produced by four marine organisms from Singapore through field testing

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Abstract. For sessile benthic organisms, the ability to produce natural antifouling biomolecules is an effective defensive strategy to avoid biofouling in nature. However, there is limited experimental evidence to evaluate these properties on local marine organisms in field testing. This study was aimed to assess the antifouling nature of crude organic extracts, prepared from four common marine species, including *Lyngbya majuscula*, *Dactylospongia* sp., *Dendronephthya* sp. and *Halimeda opuntia*. These organic extracts were incorporated into relatively stable gels and placed in the field for 28 days and then examined for biofilm coverage, invertebrate larval and algal settlement. The extracts used were in concentrations that were volumetrically equivalent to those in the living tissues of the test organisms. In addition, field testing provided a more ecologically relevant approach for determining antifouling properties of organic extracts as it ensures exposure to a natural populace of settling organisms. After 28 days of field-testing, the gel extracts from *Lyngbya majuscula* and *Dendronephthya* sp. were found to significantly reduce biofouling, while extracts from *Dactylospongia* sp., were found to significantly promote biofouling, as compared to controls. Organic extracts from *Halimeda opuntia* did not show significant differences from control plates. This study revealed that extracts deriving from *Dendronephthya* sp. and *Lyngbya majuscula* could be sources of natural antifoulants for potential biotechnological applications.

Key words. Biofilm, antifouling compounds, marine cyanobacterium, sponge, octocoral, green calcareous alga

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INTRODUCTION

Marine fouling organisms, including mussels and barnacles, are known to cause serious problems by settling on ship hulls, cooling systems of power stations and underwater structures (Nir & Reches, 2016). As a result, the economic impact of biofouling is very high. For instance, marine biofouling has directly contributed to higher fuel expenditure, higher maintenance, increased corrosion and shorter lifetime of marine infrastructure (Clare, 1998). Copper and organotin compounds, such as tributyl-tin, have been employed and have proven to be effective against these organisms (Cima & Varello, 2023). However, utilisation of such heavy-metal compounds can have significant detrimental effects in the marine ecosystem (Dafforn et al., 2008). Therefore, there is a growing urgency to investigate and develop new antifouling substances that are less lethal and more environmentally acceptable (Clare, 1998).

Many benthic marine organisms, such as algae, corals and sponges, are known to remain astonishingly free from settlement of undesirable fouling organisms despite their lack of apparent physical defences. This is due in part to the production of specialised metabolites by marine organisms as chemical defences to prevent colonisation of marine foulers (Fusetani, 2011). This would enable the survival and maintenance of living space of slow-growing sessile organisms, especially in competitive, substrata-limited communities. In fact, a recent review, covering literature from 2014 to 2020, revealed that of the 182 marine-derived antifoulants, about 44.5% were reported from marine algae and invertebrates (Liu et al., 2020). Hence, if these organisms' chemical antifouling mechanisms or substances were to be uncovered, then one could utilise these or modified related chemicals on underwater structures as antifouling paints, which would then possibly have no/minimum negative impact to the marine ecosystem.

Antifouling research has focused chiefly on toxicity and settlement inhibition assays (Davis & Wright, 1990). However, a majority of such research has been carried out in controlled laboratory settings, and their outcomes may not reliably be extrapolated to the field. This is because these experiments were conducted with homogenates or extracts from organisms reconstituted in minute volumes of artificial seawater (Rittschof et al., 1986; Sears et al., 1990; Martin & Uriz, 1993). As such, it is likely that fouling organisms may not encounter such conditions in nature. Therefore, any antifouling properties that were observed could be due to disproportionate metabolite dilutions, stagnant seawater or the extractions employed (Pawlik, 1992).

The present study investigated the antifouling nature of crude organic extracts prepared from four common marine organisms, including *Lyngbya majuscula* (a cyanobacterium), *Dactylospongia* sp. (a sponge species), *Dendronephthya* sp. (a soft coral species) and *Halimeda opuntia* (a calcareous green alga). Their organic extracts were prepared and incorporated into stable agar gels, Phytagel™, and deployed in the field to be subjected to settlement by natural populations of fouling organisms. The main objective of this study was to determine the extent of antifouling activities of extracts from four marine organisms and to test the reliability and applicability of a field-deployed antifouling assay.

MATERIAL & METHODS

A total of four marine samples were collected, namely the marine cyanobacterium, *Lyngbya majuscula*, from Pulau Hantu lagoon, the soft coral, *Dendronephthya* sp., from floating docks at Raffles Marina, a sponge, *Dactylospongia* sp., and calcareous green alga, *Halimeda opuntia*, from St. John's Island. Upon collection, samples were transported to the NIE laboratory and frozen before processing. These organisms were chosen because they appeared to be relatively devoid of fouling by macroorganisms and they represented a wide range of taxonomic taxa. Moreover, these marine organisms can be easily found throughout the year in the coastal waters of Singapore to facilitate recollections for further studies.

The method employed for sample extraction of compounds was adapted from Henrikson & Pawlik (1995). From each species, six replicates were prepared with the amount of each replicate, measured in a measuring cylinder of artificial seawater, equivalent to a volume of 35 mL. The amount of each replicate was measured to be volumetrically equivalent to the amount of gel prepared, i.e., 35 mL, so that each gel contained the natural concentration of crude extract. Replicates were chopped into smaller pieces and extracted two times with 100 ml of methanol in each extraction round. The sample mixtures were filtered, and the organic solvent removed in vacuo using rotary evaporator. The dried organic extracts from each replicate were stored in a –80°C freezer for later use.

Gels were made by adding 1.52 g of Phytagel™ (Sigma Chemical) to 32 mL distilled water. The gel mixture was heated in a microwave oven until boiling. Regular mixing was required to ensure all the Phytagel™ was properly dissolved. The mixture was allowed to cool slightly before an aliquot of extract dissolved in 3 mL of distilled water was added and stirred. The mixture was then poured into a 9 cm diameter circular Petri dish, with a surface area of 63.64 cm^2. The gels were left to cool and harden before being stored in a refrigerator for later use. Similar formulation was also used in several ecological studies on the antifouling investigations of marine organisms, including Pereira et al. (2002), da Gama et al. (2003), Barbosa et al. (2007), da Gama et al. (2008), Tan et al. (2010), da Gama et al. (2022) and Jasim et al. (2022). The Phytagel™ method was first reported by Henrikson & Pawlik (1995) with further modification by da Gama et al. (2002). The main benefits of this method are that it allows organic extracts to be introduced in the gel matrix at the natural volumetric amounts found in the marine organisms and its slow diffusion in the water column (da Gama et al., 2002). In addition, the relatively higher concentration of Phytagel™ used in the formulation is to allow maintenance of the gel shape in the petri dish due to a prolonged field assay duration of over 4 weeks.

Six replicates of each extract treatment were prepared in addition to six positive gel controls that contained no organic extracts. The plated gels were placed in a randomised order within a cage using a solid stainless-steel frame, stainless steel wire mesh and plastic cable ties. The stainless-steel structure with the gels was hung securely within a boating berth 1 m below the water surface and secured with nylon ropes at Raffles Marina, Singapore in September 2009. The water salinity was 25% and the water temperature was 28°C. Lead weights were added to the bottom of the stainless-steel structures to ensure that the structures were in a stable position and parallel to tidal flow.

Settlements by microbial biofilm, invertebrates and algae on the surface of each gel plate were measured on a weekly basis using a dot-grid estimate method (Foster et al., 1991; Meese & Tomich, 1992). The outline of the gel was traced onto clear transparency film and the area within was marked in a dot grid with all points 0.5 cm apart. Area cover was calculated by dividing the recorded points by the total number of points and multiplying it by the surface area of the gel (in cm^2). In addition to area cover, individual barnacles growing on gels were recorded after 28 days using a dissecting microscope to determine barnacle abundance.

Data collected were tested for homogeneity of variance using Bartlett's Test and Levene's Test. A one-way Analysis of Variance (ANOVA) was used to analyse the mean area of biofilm against different treatments on week 1. A two-way ANOVA was used to analyse the combined mean area cover of invertebrates and algae against different treatments in weeks 3 and 4 of field testing. Finally, one-way ANOVA was used to analyse the barnacle abundance against different treatments on week 4. Non-significant higher order interaction terms were systemically discarded until only significant terms remained in the model for subsequent interpretation. Tukey's post-hoc test was used to further analyse any significant differences observed (Zar, 1999). All statistical analyses were conducted using MINITAB© (version 15) software.

RESULTS

Area cover of biofilm was recorded on both control and treatment gel plates from week 1 to 4 (Fig. 1). There were significant differences in mean area cover in week 1 among treatments (ANOVA, $p<0.05$, $F=49.75$, $df=4$). Tukey's post-hoc test (Tukey-HSD) revealed that gel plates containing extracts of *Halimeda opuntia*, *Lyngbya majuscula* and *Dactylospongia* sp. had a significant increase in biofilm formation compared to controls in week 1 ($p<0.05$) while gels containing extracts of soft coral, *Dendronephthya* sp., had reduced biofilm formations as compared with controls in week 1 ($p<0.05$). In weeks 2 to 4, the biofilm coverage for all treated gel plates were not significantly different from control gel plates in their respective weeks (Fig. 1).

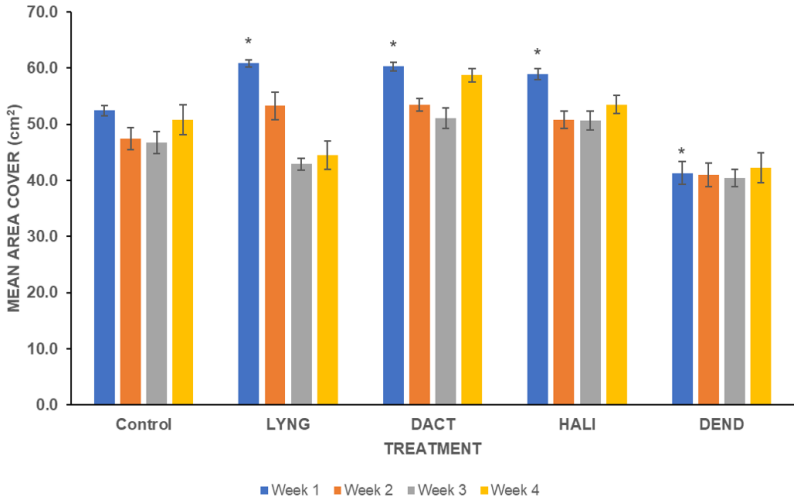

Fig. 1. Mean area cover of biofilm on gel plates over four weeks of exposure. All bars are mean values of six replicates, vertical bars indicate standard error. Treatments were as follows: Control = Positive control gel, LYNG = *Lyngbya majuscula*, DACT = *Dactylospongia* sp., HALI = *Halimeda opuntia* and DEND = *Dendronephthya* sp. *Tukey's post-hoc test revealed that plates containing extracts of HALI, LYNG and DACT had significantly higher biofilm cover while plates with DEND extracts had significantly lower biofilm compared to Controls, respectively in week 1 ($p<0.05$).

Mean area cover by fouling invertebrates and algae became more obvious in both control and treatment plates after week 3 and increased steadily to week 4 where the highest area cover was recorded (Fig. 2). Two- way ANOVA analysis on mean area cover crossed with factors, treatments and field testing periods (week 3 and week 4), indicated no significant interactions between treatments and field testing periods ($p>0.05$). There were significant differences in mean area cover among treatments (ANOVA, $p < 0.05$, $F=14.88$, $df = 4$). In addition, there were significant differences in mean area cover among field testing periods (ANOVA, $p<0.05$, $F=3.99$, $df=1$). Tukey's post-hoc test (Tukey-HSD) revealed that gels containing extracts of *Lyngbya majuscula* and *Dendronephthya* sp. reduced settlement of invertebrates and algae significantly compared to controls ($p<0.05$), while gel plates containing extracts of *Dactylospongia* sp. promoted settlement of invertebrates and algae compared to controls ($p<0.05$). Gel plates containing extracts of *Halimeda opuntia* showed no significant differences in settlement of invertebrates and algae from the control plates ($p>0.05$).

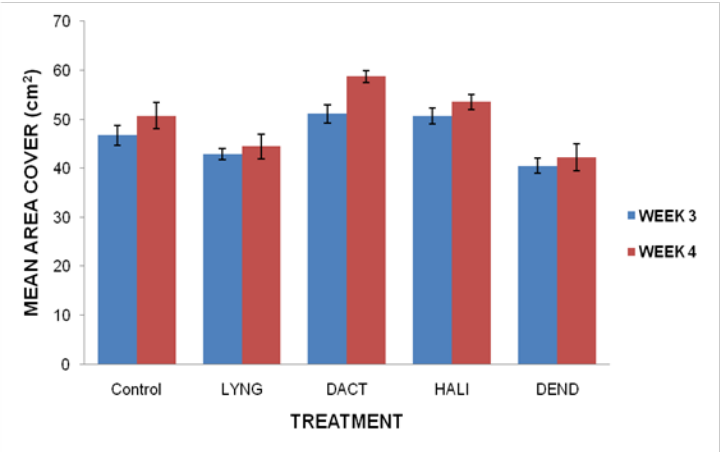

Fig. 2. Mean area cover of invertebrates and algae on gel plates on week 3 and 4. All bars are mean values of six replicates, vertical bars indicate standard error. Treatments were as follows: Control = Positive control gel, LYNG = *Lyngbya majuscula*, DACT = *Dactylospongia* sp., HALI = *Halimeda opuntia* and DEND = *Dendronephthya* sp. Tukey's post-hoc test revealed that LYNG and DEND extracts significantly reduced fouling while DACT extracts significantly increased fouling compared to controls, respectively ($p<0.05$).

The most common invertebrate foulers that settled on control and treatment gel plates were the larvae of barnacles (Fig. 3). There were significant differences in barnacle abundance among treatments after week 4 (ANOVA, $p<0.05$, $F = 244.20$, $df = 4$). Tukey's post-hoc test (Tukey-HSD) revealed that gel plates containing extracts of *Lyngbya majuscula* and *Dendronephthya* sp. had significantly lower barnacle counts ($p<0.05$), while gel plates containing extracts of *Dactylospongia* sp. had significantly higher barnacle count ($p<0.05$). Gel plates containing extracts of *Halimeda opuntia* showed no significant differences in barnacle counts from control plates ($p>0.05$).

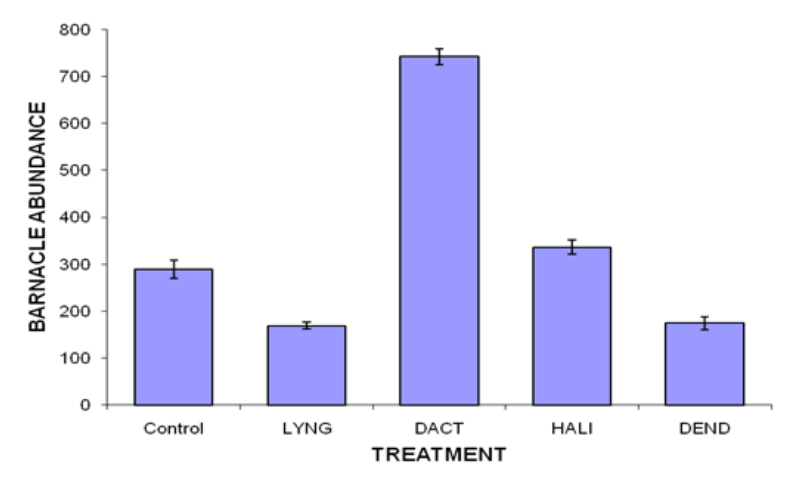

Fig. 3. Individual barnacle counts settling on gel plates on week 4. All bars are mean values of six replicates, vertical bars indicate standard error. Treatments are as follows: Control = Positive control gel, LYNG = *Lyngbya majuscula*, DACT = *Dactylospongia* sp., HALI = *Halimeda opuntia* and DEND = *Dendronephthya* sp. Tukey's post-hoc test indicated that LYNG and DEND extracts significantly inhibited barnacle settlement while DACT significantly promoted settlement compared to Controls ($p<0.05$).

DISCUSSION

The present study investigated the antifouling nature of organic extracts derived from four marine species of diverse taxonomic groups, namely *Lyngbya majuscula*, *Dactylospongia* sp., *Halimeda opuntia* and *Dendronephthya* sp., using ecologically relevant approaches. Gel plates containing extracts of *Dendronephthya* sp. were found to have significantly reduced biofilm formation, especially in week 1, and reduced settlement of invertebrates and algae. We also found that gel plates containing extracts of *L. majuscula* had higher biofilm coverage in the early stages of submergence (week 1) compared to control plates. However, beyond week 1, the biofilm coverage on plates containing marine cyanobacterial extracts were not significantly different from control plates of the respective weeks. In addition, plates containing *L. majuscula* extracts were found to have lower settlement of invertebrates and algae in the later stages of submergence (weeks 3 and 4) compared with control plates. These observations seem to suggest that the marine cyanobacterial extracts could affect both biofilm formation and settlement of macrofoulers. These results proved to be interesting because even though extracts from both the marine organisms were found to have the ability to reduce settlement of macroorganisms, they affected biofilm formation differently. These findings indirectly suggest that even though biofilm formation is known to play an important role in the development of trophic succession on benthic micro systems, they can influence the settlement of subsequent marine macrofoulers in varied ways (Egan et al., 2001). Factors that promote the growth of biofilm can positively influence certain species of marine organisms to form symbiotic relationships with film-formers in the habitat (Egan et al., 2001). This symbiotic relationship results in a protective cover for certain marine organisms that prevents them from overgrowth by natural competitors within the settling communities (Egan et al., 2001).

Other studies suggest that certain bacterial biofilm produce and release antifouling compounds that repel fouling organisms (Armstrong et al., 2001) or influence the normal development and survival of some vertebrates and algae (Egan et al., 2001). This may be the case in treatments containing extracts of *Dendronephthya* sp., where a specific bacterial biofilm could be present on the plates which caused a reduction in settlement of invertebrates and algae. Studies by Dobretsov & Qian (2004) found that specific bacteria associated with the soft coral *Dendronephthya* sp. may contribute to reduced fouling, as the bacteria produce antifouling compounds that work against both biofilm formation and settlement of invertebrates and algae on the surface of their host (Harder et al., 2003; Dobretsov & Qian, 2004). Future works would include comparing the nature of the microbial community associated with the biofilm on gel plates containing extracts of *Dendronephthya* sp. and *Lyngbya majuscula* as well as controls. The chemical nature of antifouling specialised metabolites produced by these local marine organisms and associated microbes would be of interest as well. Understanding the chemistry and mechanism of such substances would be useful in the production of antifouling coatings for biotechnological applications. Several antifouling compounds have been reported from these two marine species collected from other locations. A research group in Japan identified four new antifouling seco-steroids, isogosterones A–

D from *Dendronephthya* sp. obtained from the Izu Peninsula, Japan (Tomono et al., 1999). The alkaloid, trigonelline, a *N*-methylated niacin analog, was isolated from *Dendronephthya* sp. from Chichijima in the Ogasawara Islands, Japan. This compound exhibited the same level of settling-inhibitory activity against the acorn barnacle *Balanus amphitrite* larvae as $CuSO_4$ (Kawamata et al., 1994). Mixtures of sterols and fatty acids prepared from another collection of *Dendronephthya* sp. off Chichijima and Hahajima, Japan, showed significant antifouling activity against the blue mussel, *Mytilus edulis* (Mizobuchi et al., 1993). Further purification of this mixture led to the isolation of the sterol, β-sitosterol, which had the highest antifouling activity compared to cholesterol and β-cholestanol. A series of compounds belonging to the hybrid peptide-polyketide structure class with antifouling activity have been reported from *L. majuscula* (Tan et al., 2010). The most active antifouling molecule, dolastatin 16, appeared to be weakly toxic to *Balanus amphitrite* cyprids with a LC_{50}/EC_{50} value of > 6000. Lastly, three new cyclic depsipeptides, tiahuramides A–C, isolated from a Tahitian collection of *L. majuscula* inhibited the growth of three marine biofouling pathogenic bacteria, *Aeromonas salmonicida*, *Vibrio anguillarum* and *Shewanella baltica*, with MIC values ranging from 7.0 to 33.0 μM (Levert et al., 2018).

There is much literature describing the different antifouling properties of natural products obtained from marine organisms (Qian et al., 2015; Liu et al., 2020). However, there is still a lack of field experimental evidence to explain the ecological functions of these compounds (Pawlik, 1992). The field method used in this study may provide an ecologically relevant approach to test for antifouling properties (Henrikson & Pawlik, 1995). Most studies to date employ the application of extracts directly onto panels (Davis & Wright, 1990). This creates problems of pooling which may alter surface characteristics. The incorporation of compounds into a gel matrix in this study may represent a better alternative method, as it would be possible to add the extracts/compounds into the gels at natural volumetric concentrations, hence ensuring an even spread on and within the settlement surface (Henrikson & Pawlik, 1995).

Since this technique involved field testing, it also eliminates problems associated with unrealistic water flow, uneven compound concentrations and exposure to only one or a few species of fouling organisms. As the gels remained relatively unchanged throughout the whole period of field testing, they could be suitable substrata for larval and algal propagules in long field assays. Living marine organisms may concentrate their antifouling compounds at different portions of their anatomy (Henrikson & Pawlik, 1995). This assaying technique can provide a more conservative method of assessing antifouling properties, as the extracts are distributed homogeneously within the gels. According to Henrikson & Pawlik (1995), the technique of using gels made of Phytagel™ has an added advantage because the sugar residues of the gel matrix bind strongly to the organic compounds and results in a slower rate of extracts diffusing into the seawater. The reduced rate of compound diffusion would allow gels to be left in the field longer to facilitate data collection. Moreover, this assaying technique can serve as a standard method, which can be modified to incorporate pure compounds and other crude extracts in varying concentrations into the gels to test for antifouling properties in a dose dependent manner.

Since the introduction of the Phytagel™ method by Henrikson & Pawlik (1995), a number of chemical ecology studies have adopted this gel-based method to evaluate the allelopathic effects of marine natural products. For instance, the sponge-derived clionapyrrolidine A, when incorporated into Phytagel™ at close to natural volumetric concentration, killed coral tissue when in direct contact with live coral for periods of 1–4 days (Chaves-Fonnegra et al., 2008). A study by Andras et al. (2012) showed the allelopathic effect of four common seaweed extracts, embedded in Phytagel™, to induce bleaching on natural colonies of *Porites rus* in field testing. The demonstration of induced allelopathy in a seaweed, or of competitors reducing seaweed chemical defences against herbivores was carried out using the gel method (Rasher & Hay, 2014). In addition, the allelopathic effects of direct contact between marine cyanobacterial extracts and the hard coral, *Porites porites*, was assessed by the Phytogel™ method (Puyana et al., 2019). Other allelopathic studies using the gel method include the role of sponge-derived b-sitosterol in preventing the overgrowth of aggressive zoanthids, *Zoanthus sansibaricus*, by affecting its symbionts (Singh & Thakur, 2021), the identification of macroalgal terpenes as potent allelopathic agents against reef corals (Rasher et al., 2011) and the structuring effects of natural products from the sea fan, *Phyllogorgia dilatate*, on benthic communities (Ribeiro et al., 2017). The Phytagel™ method has also been used to demonstrate antifouling properties of marine-derived extracts/compounds. This includes antifouling studies on seaweeds (da Gama et al., 2002; da Gama et al., 2008; Schwartz et al., 2017; Jasim et al., 2022), marine benthic invertebrates (Engel & Pawlik, 2000; Mora-Cristancho et al., 2011; Angulo-Preckler et al., 2015; Angulo-Preckler et al., 2020) and marine-derived specialised metabolites (Yang et al., 2006; Tan et al., 2010). Although the gel method is an ecologically relevant approach to testing of antifouling activities, for the purpose of evaluating compounds for potential industrial application and upscaling, the coating method would provide more realistic results (Lim et al., 2014).

In conclusion, the present study involving incorporation of marine-derived compounds into Phytagel™, coupled with field testing, is a useful and reliable approach in evaluating the antifouling activities of extracts of marine organisms in an ecologically relevant manner. In addition, crude extracts from the marine cyanobacterium, *Lyngbya majuscula*, and soft coral, *Dendronephthya* sp., showed the most promising results in terms of antifouling properties. Future works include the identification of specialised metabolites responsible for the antifouling properties as well as comparing the nature of microbial communities on gel plates containing extracts of *Dendronephthya* sp. and *Lyngbya majuscula* with untreated controls.

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LITERATURE CITED

Andras TD, Alexander TS, Gahlena A, Parry RM, Fernandez FM, Kubanek J, Wang MD & Hay ME (2012) Seaweed allelopathy against coral: surface distribution of a seaweed secondary metabolite by imaging mass spectrometry. Journal of Chemical Ecology, 38: 1203–1214.

Angulo-Preckler C, Cid C, Oliva F & Avila C (2015) Antifouling activity in some benthic Antarctic invertebrates by "in situ" experiments at Deception Island, Antarctica. Marine Environmental Research, 105: 30–38.

Angulo-Preckler C, García-Lopez E, Figuerola B, Avila C & Cid C (2020) Natural chemical control of marine associated microbial communities by sessile Antarctic invertebrates. Aquatic Microbial Ecology, 85: 197–210.

Armstrong E, Yan L, Boyd KG, Wright PC & Burgess JG (2001) The symbiotic role of marine microbes on living surfaces. Hydrobiologia, 461: 37–40.

Barbosa JP, Fleury BG, da Gama BAP, Teixeira VL & Pereira RC (2007) Natural products as antifoulants in the Brazilian brown alga *Dictyota pfaffii* (Phaeophyta, Dictyotales). Biochemical Systematics and Ecology, 35: 549–553.

Chaves-Fonnegra A, Castellanos L, Zea S, Duque C, Rodríguez J & Jiménez C (2008) Clionapyrrolidine A - a metabolite from the encrusting and excavating sponge *Cliona tenuis* that kills coral tissue upon contact. Journal of Chemical Ecology, 34: 1565–1574.

Clare AS (1998) Towards non-toxic antifouling. Journal of Marine Biotechnology, 6: 36.

Cima F & Varello R (2023) Potential disruptive effects of copper-based antifouling paints on the biodiversity of coastal macrofouling communities. Environmental Science and Pollution Research International, 30: 8633–8646.

da Gama BAP, Pereira RC, Carvalho AGV, Coutinho R & Yoneshigue-Valentin Y (2002) The effects of seaweed secondary metabolites on biofouling. Biofouling, 18: 13–20.

da Gama BAP, Pereira RC, Soares AR, Teixeira VL & Yoneshigue-Valentin Y (2003) Is the mussel test a good indicator of antifouling activity? A comparison between laboratory and field assays. Biofouling, 19(Supplement): 161–169.

da Gama BAP, Carvalho AGV, Weidner K, Soares AR, Coutinho R, Fleury BG, Teixeira VL & Pereira RC (2008) Antifouling activity of natural products from Brazilian seaweeds. Botanica Marina, 51: 191–201.

Dafforn KA, Glasby TM & Johnston EL (2008) Differential effects of tributyltin and copper antifoulants on recruitment of non-indigenous species. Biofouling, 24: 23–33.

Davis AR & Wright AE (1990) Inhibition of larval settlement by natural products from the ascidian, *Eudistoma olivaceum* (Van Name). Journal of Chemical Ecology, 16: 1349–1357.

Dobretsov S & Qian PY (2004) The role of epibotic bacteria from the surface of the soft coral *Dendronephthya* sp. in the inhibition of larval settlement. Journal of Experimental Marine Biology and Ecology, 299: 35–50.

Egan S, James S, Holmström C & Kjellerberg S (2001) Inhibition of algal spore germination by the marine bacterium *Pseudoalteromonas tunicata*. FEMS Microbiology Ecology, 35: 67–73.

Engel S & Pawlik JR (2000) Allelopathic activities of sponge extracts. Marine Ecology Progress Series, 207: 273–281.

Foster MS, Harrold C & Hardin DD (1991) Point versus photo quadrat estimates of the cover of sessile marine organisms. Journal of Experimental Marine Biology and Ecology, 146: 193–203.

Fusetani N (2011) Antifouling marine natural products. Natural Product Reports, 28: 400–410.

Harder T, Lau SCK, Dobretsov S, Fang TK & Qian PY (2003) A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. FEMS Microbiology Ecology, 43: 337–347.

Henrikson AA & Pawlik JR (1995) A new antifouling assay method: results from field experiments using extracts of four marine organisms. Journal of Experimental Marine Biology and Ecology, 194: 157–165.

Jasim SA, Jabr HS, Karim YS, Supaeva A, Kadhim AJ, Jabbar AH, Yasin G, Jalil AT & Mustafa YF (2022) Fouling resistance investigations of seaweed products. Brazilian Journal of Biology, 84: e263386.

Kawamata M, Kon-ya K & Miki W (1994) Trigonelline, an antifouling substance isolated from an octocoral *Dendronephthya* sp. Fisheries Science, 60: 485–486.

Levert A, Alvariño R, Bornancin L, Abou Mansour E, Burja AM, Genevière AM, Bonnard I, Alonso E, Botana L & Banaigs B (2018) Structures and activities of tiahuramides A-C, cyclic depsipeptides from a Tahitian collection of the marine cyanobacterium *Lyngbya majuscula*. Journal of Natural Products, 81: 1301–1310.

Lim CS, Lee SSC, Leong W, Xian YN & Teo SLM (2014) A short review of laboratory and field testing of environmentally benign antifouling coatings. Indian Journal of Geo-Marine Sciences, 43: 2067–2074.

Liu LL, Wu CH & Qian PY (2020) Marine natural products as antifouling molecules – a mini-review (2014–2020). Biofouling, 36: 1210–1226.

Martin D & Uriz MJ (1993) Chemical bioactivity of Mediterranean benthic organisms against embryos and larvae of marine invertebrates. Journal of Experimental Marine Biology and Ecology, 173: 11–27.

Meese RJ & Tomich PA (1992) Dots on the rocks: a comparison of percent cover estimation methods. Journal of Experimental Marine Biology and Ecology, 165: 59–73.

Mizobuchi S, Shimidzu N, Katsuoka M, Adachi K & Miki W (1993) Antifouling substances against the mussel in an octocoral *Dendronephthya* sp. Nippon Suisan Gakkaishi, 59: 1195–1199.

Mora-Cristancho JA, Arévalo-Ferro C, Ramos FA, Tello E, Duque C, Lhullier C, Falkenberg M & Schenkel EP (2011) Antifouling activities against colonizer marine bacteria of extracts from marine invertebrates collected in the Colombian Caribbean Sea and on the Brazilian coast (Santa Catarina). Zeitschrift für Naturforschung C, 66: 515–526.

Nir S & Reches M (2016) Bio-inspired antifouling approaches: the quest towards non-toxic and non-biocidal materials. Current Opinion in Biotechnology, 39: 48–55.

Pawlik JR (1992) Chemical ecology of the settlement of benthic marine invertebrates. Oceanography and Marine Biology: An Annual Review, 30: 273–335.

Pereira RC, Carvalho AGV, da Gama BAP & Coutinho R (2002) Field experimental evaluation of secondary metabolites from marine invertebrates as antifoulants. Brazilian Journal of Biology, 62: 311–320.

Puyana M, Prato JA, Nieto CF, Ramos FA, Castellanos L, Pinzón P & Zárate JC (2019) Experimental approaches for the evaluation of allelopathic interactions between hermatypic corals and marine benthic cyanobacteria in the Colombian Caribbean. Acta Biológica Colombiana, 24: 243–254.

Qian PY, Li Z, Xu Y, Li Y & Fusetani N (2015) Mini-review: Marine natural products and their synthetic analogs as antifouling compounds: 2009–2014. Biofouling, 31: 101–122.

Rasher DB, Stout EP, Engel S, Kubanek J & Hay ME (2011) Macroalgal terpenes function as allelopathic agents against reef corals. Proceedings of the National Academy of Sciences, USA, 108: 17726-17731.

Rasher DB & Hay ME (2014) Competition induces allelopathy but suppresses growth and anti-herbivore defence in a chemically rich seaweed. Proceedings of the Royal Society B: Biological Sciences, 281(1777): 20132615.

Ribeiro FV, da Gama BA & Pereira RC (2017) Structuring effects of chemicals from the sea fan *Phyllogorgia dilatata* on benthic communities. PeerJ, 5: e3186.

Rittschof D, Hooper IR & Costlow JD (1986) Barnacle settlement inhibitors from sea pansies, *Renilla reniformis*. Bulletin of Marine Science, 39: 376–382.

Schwartz N, Dobretsov S, Rohde S & Schupp PJ (2017) Comparison of antifouling properties of native and invasive *Sargassum* (Fucales, Phaeophyceae) species. European Journal of Phycology, 52: 116–131.

Sears MA, Gerhart DJ & Rittschof D (1990) Antifouling agents from marine sponge *Lissodendoryx isodictyalis* Carter. Journal of Chemical Ecology, 16: 791–799.

Singh A & Thakur NL (2021) Allelopathic interaction among rocky intertidal invertebrates: sponge *Cinachyrella* cf. *cavernosa* and Zooxanthellate zoanthids *Zoanthus sansibaricus*. Hydrobiologia, 848: 4647–4659.

Tan LT, Goh BPL, Tripathi A, Lim MG, Dickinson GH, Lee SSC & Teo SLM (2010) Natural antifoulants from the marine cyanobacterium *Lyngbya majuscula*. Biofouling, 26: 685–695.

Tomono Y, Hirota H, Imahara Y & Fusetani N (1999) Four new steroids from two octocorals. Journal of Natural Products, 62: 1538–1541.

Yang LH, Lee OO, Jin T, Li XC & Qian PY (2006) Antifouling properties of 10β-formamidokalihinol-A and kalihinol A isolated from the marine sponge *Acanthella cavernosa*. Biofouling, 22: 23–32.

Zar JH (1999) Biostatistical Analysis 4th edition. Prentice-Hall International, New Jersey, 663 pp.