

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

Robert A. Bara^{1*}, Fitje Losung¹, Calvyn F. Sondak¹, Jimmy Posangi², Remus Maradou¹, Stefan Kehraus³

¹Faculty of Fisheries and Marine Science Sam Ratulangi University Manado Indonesia Jl. Kampus Bahu Unsrat 95115 Manado Indonesia

²Faculty of Medicine Sam Ratulangi University Manado Indonesia Jl. Kampus Bahu Unsrat 95115 Manado Indonesia

³Institute of Pharmaceutical Biology Bonn University Bonn Nußallee 653115 Bonn, Germany

ABSTRACT

Marine fouling is attaching organisms to objects floating or submerged in seashores, such as ships, docks, and offshore structures. The impact caused is the reduced lifespan of the thing attached to the biofouling. In addition, there was a spread of invasive species. The government and industries in the United States spend over 6.5 million dollars annually on their budget to overcome this problem. So far, the prevention of marine biofouling is using antifouling paint on ships and submerged structures. One of them is organotin tributyltin (TBT) proven effective for preventing fouling; however, it is not environmentally friendly.

Sponge *Siphonodictyon coralliphagum* growing in Salibabu Island waters, Talaud Islands, with previously shown activities against prokaryotes, was collected and extracted by maceration using ethanol as a solvent. The extract was then added to the base paint with several compositions of 5%, 10%, and 25% applied to the paving block surface. Positive control uses paint with copper additives. The objects are then immersed in the seashore below subtidal depths. The observation was carried out every seven days until day 28. The paving block was observed on day 90 after immersion.

Significant results were obtained where macrofouling was only able to grow in negative control and positive control. The extract sponge *S. coralliphagum* has vigorous antifouling activity against marine biofouling. Furthermore, the extract was fractionated using liquid chromatography and continued by testing their activity against Gram-positive and negative bacteria. Further investigation of NMR spectra from the active fraction to prokaryotic cells shows a cyclic aliphatic compound with several keto-enol functional and methyl aliphatic groups on their chain.

KEYWORDS: Biofouling, sponge; *Siphonodictyon coralliphagum*; antifouling activity, NMR, Liquid phase chromatography.

ARTICLE DETAILS

Published On:
13 March 2024

Available on:
<https://ijpbms.com/>

INTRODUCTION

Marine fouling is the attachment process of marine organisms to objects that float or are submerged in the sea, such as ships, docks, and offshore structures. It has many negative impacts on manufactured marine systems, such as loading excess, structures occlusion, increased drag coefficient, corrosion, and reduced lifespan of the object attached to the biofouling organism. Prevention of fouling requires enormous costs, as reported by [1]; the US government and industry spend at least 6.5 million dollars per annum to overwhelmed this problem, while the direct effect of fuel consumption increases

up to 30 billion dollars per annum affected by this organism. On ships, biofouling will increase the weight of the vessel and the hydrodynamic drag and reduce the ship's maneuverability, causing an increase in costs through increased use of labor, fuel, and docking time [2]. Biofouling significantly impacts the cost of structural inspection and maintenance because of the importance of colonization due to favorable environmental parameters (i.e., the warmer temperature of seawater, intense action of the waves generating mixing of the nutritional elements, as well as strong light). It is a complex phenomenon involving a

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

diversity of marine species which constitute communities whose growth dynamic is driven by physical and biological processes [3].

Marine is an environment rich in biological resources, inspiring humans to explore and exploit to get a variety of biological materials as natural products from the results of primary and secondary metabolites in the form of bioactive substances from marine biota. Various marine organisms in Indonesian waters were investigated for their bioactivity, including algae, soft corals, mollusks, crustaceans, echinoderms, sponges, and ascidians. The use of marine resources, especially in natural products, has only developed from the past until recent years [4-6]. These natural products are commonly used in the pharmaceutical industry and the environment.

The sponge is one of the marine organisms that have various bioactive substances. More than 6,000 new compounds were isolated from marine organisms, and 33% were isolated from sponges [4, 7]. The sponge-derived bioactive compounds are reported with pharmacological activities or effects, including antiviral, antibacterial, cytotoxic, and anti-inflammatory [8].

Sponges are also reported to have activities that play a role in ecology; one is antifouling. Several research groups reported antifouling activity obtained from sponges [8-13]. Previously, the *Siphonodictyon coralliphagum*, A marine sponge species growing in Salibabu Beach, showed potent antibacterial activity against a series of Gram-positive and negative bacteria [14].

MATERIALS AND METHODS

Sampling

Sponges of *S. coralliphagum* (Figure 1) taken at Talaud Islands Waters (Salibabu Island) at coordinates 3 ° 51'54.6 "N 126 ° 41'17.1" E (Figure 2) using SCUBA. The collected samples were put in plastic bags, labeled, and photo documented for further identification. Sponge identification refers to the website

<http://www.wetwebmedia.com/SWPOTD556.htm> and the world register of marine species on the website <http://www.marinespecies.org/index.php>. Sponge samples were confirmed [15].

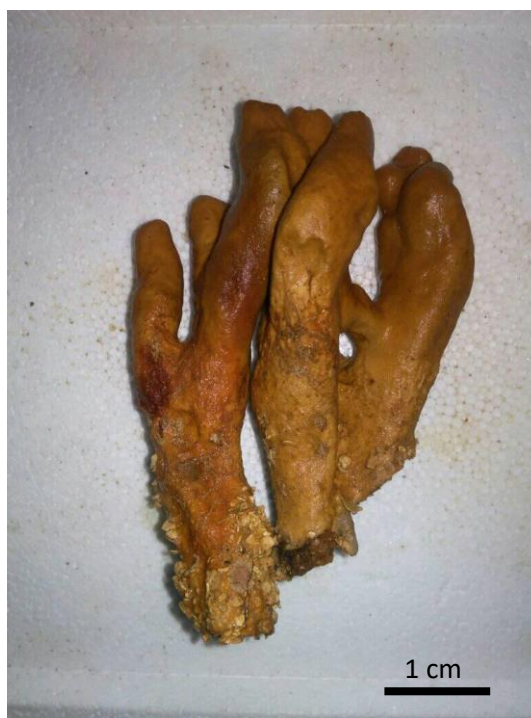


Figure 1. Sponge *Siphonodictyon coralliphagum*

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*



Figure 2. Sponge sampling location *S. coralliphagum*, Salibabu Island, Talaud Islands Regency, Indonesia. Blue dot = sampling point.

Extraction

The sponge sample of *S. coralliphagum*, collected from the Salibabu Waters, was extracted using ethanol as a solvent using the maceration technique. Previously, the sample was washed using fresh water to reduce the salt content from the sponge body. The sponge was sliced, put into a PTE plastic bottle, and soaked in ethanol for 7x24 hours. The extract was then decanted and evaporated using Rotary Vacuum Evaporator. The extract was weighed before being dissolved with 98% ethanol for further analysis.

Antifouling activity testing

Antifouling activity testing was carried out using the method described in Moninka (2000) with some modification [2, 16, 17] which was done by painting on the top surface of a 10.5 x 21 cm concrete paving block with the Avianpaint™ brand as a base layer. After the base layer is dried, ethanolic sponge extract *S. coralliphagum* is mixed with the paint above with increased concentrations of 5 (further documented as treatment 1), 10 (further documented as treatment 2), and 25% w/v (further documented as treatment 3), as treatments. Extract suspension was then re-applied to the surface of the paving block with three replications in each treatment. The negative control is applied using the same paint without adding extract. Positive control was using Nipponpaint™

copper antifouling paint. The paint on the paving block was allowed to dry for three days. Paving blocks were placed in a subtidal area where the objects were completely submerged in seawater during the experiment. The antifouling activity test was conducted at coordinates 1° 26'36.7" LU and 125° 12'8.4" East (Figure 3). The study was conducted for three months, with intensive data collection every seven days in the first month of testing. Data was collected on the 7th, 14th, 21st, and 28th days after laying on a subtidal area. The following paving-block tests were left for 60 days to observe biofouling growth. The observation was stopped on the 90th day to see the attachment process and development of macro-foulers. Data is collected by following the treated paving surfaces. Microscopic observations were made using a Digital Microscope Zoom® by observing the formation of a biofilm layer on the surface of a paving block. Observation of paving surface scrapings was done by smearing the scrapped objects on the surface of the object-glass; smear results were burned on fire and soaked in a 10% methylene blue solution for 10 minutes, then the object glasses were allowed to dry. Paving blocks were submerged in the subtidal area until the 90th day to analyze the macro fouler's development. Data collection for the last day was done by counting the number of fouling organisms attached to the paving surface in each treatment.

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*



Figure 3. *In situ* testing location, Harbor of the Indonesian Navy Patrol, Bitung, Indonesia. Red dot = *in situ* location.

Fractionation

Fractionation of sponge extract *S. coralliphagum* performed by using the Vacuum Liquid Chromatography (VLC) method using the solvent hexane, dichloromethane: ethyl acetate; ethyl acetate: methanol to obtain 11 fractions with 500 ml each eluent. Furthermore, each obtained fraction was dried. The obtained fractions were tested for their activity against the Gram-positive marine isolates *Bacillus megaterium* DSM32T and Gram-negative *Escherichia coli* DSM498.

Antimicrobial Assays

The fractions obtained in the previous procedure were individually tested against *Bacillus megaterium* DSM 32^T and *Escherichia coli* DSM 498 using Agar Diffusion Assay for antimicrobial assays. The strains were grown in 10 ml slope nutrient agar and incubated at 37° C overnight. The cultures were harvested, and their density was measured. The initial OD₆₀₀ was set to 1 to reach a concentration of 8x10⁸ cells/ml. The cultures were diluted to 8x10⁶ cells/ml, and 1 ml of the bacterial culture was subjected and seeded into the Luria Betani Agar (LB Agar). After solidification, 20 µl of the sponge fractions were spotted on the plates. Chloramphenicol (1mg/ml) and 96% ethanol for 20 µL each served as positive and negative controls, respectively. All samples were performed in triple. Plates were incubated at 37°C for 18 h and checked for inhibition zones.

NMR experiments

NMR experiments were performed at 25°C in a Bruker Avance DRX 300 MHz spectrometer with an indirect 5 mm triple TBI 1H/[9]/13C probe head using standard pulse sequences available in the Bruker software. The samples were dissolved in 700 µL of 99.8% MeOD. 1D 1H spectra were recorded at 300 Mhz with a 30° pulse, a delay D1 of 2s and 64 scans. As an external standard, chemical shifts were expressed in ppm relative to TMS (Tetrametylsilane). Double-quantum filtered 1H-1H correlated spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond coherence (HMBC) with a 60-ms mixing time were performed according to standard pulse sequences to assign ¹H and ¹³C resonances.

RESULT AND DISCUSSION

Antifouling observations

Observations from *in-situ* testing after subtidal submersion were presented in Figure 4. Seven days after submersion, the paving surfaces looked relatively clean, and no fouling organisms were observed in the paving blocks of each treatment or both controls.

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

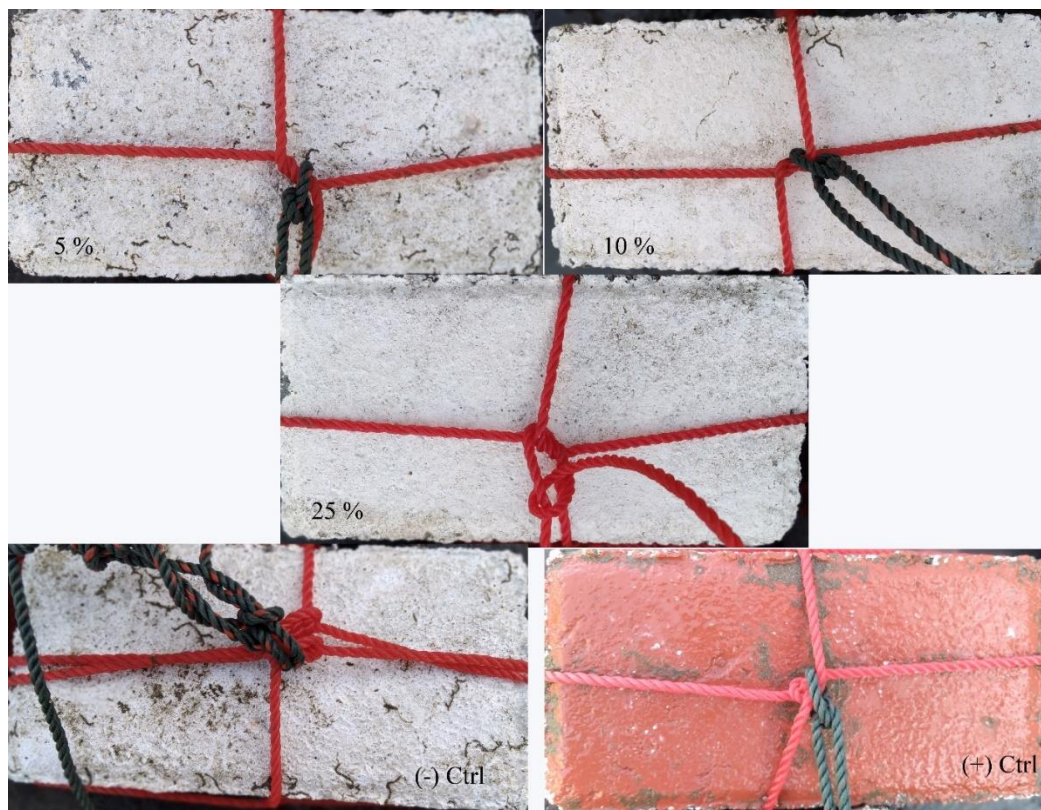


Figure 4. Display of paving blocks with different treatments after seven days of subtidal immersion.

The results of the 14th-day observations are shown in Figure 5. At 14 days after subtidal submersion, no macroscopic organisms were attached to the paving test in each treatment and in both controls. Marine biofouling commonly starts

growing after several weeks until months of submersion [2, 3, 18]. Observation on the 14th day saw sediment deposition on the surface of the test paving block due to the high sedimentation process in the Harbor Port of Bitung.



Figure 5. Display of paving blocks with different treatments after 14 days of subtidal immersion.

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

Microscopic observations of the 14th day of submerged paving blocks of the scraped paving surface showed the fiber-like structure produced by bacteria observed in treatments 1 (5%) and 2 (10%) of sponge extracts and negative control. It is likely the initial formation of a biofilm from bacteria. The distribution of fiber-like structure formation was observed to decrease with an increasing percentage of the extract on paving blocks. The scraped paving surface in treatment 3 (25%) and positive control did not show the presence of hyphae threads produced by bacteria-producing hyphae such as Actinomycetes (Figure 6). Biofilm formation is a complex process with several stages [19, 20]. The procedure typically

starts with the settlement of bacteria on the submerged surface. From here, a conditioning film is formed, which involves the adsorption of organic compounds (i.e., proteins, carbohydrates, and nucleic acids) to the surface [21, 22]. The following stage in biofilm formation is the attachment of cells to the surface. Afterward, cell proliferation leads to the formation of a micro-colony, and cell-cell chemical signaling activates biofilm genes which enhance micro-colonies ability to form and adhere to the solid interfaces. Established biofilms can then detach from the tangible interfaces to facilitate the multiplication and distribution of cells and biofilm microcolonies [23].

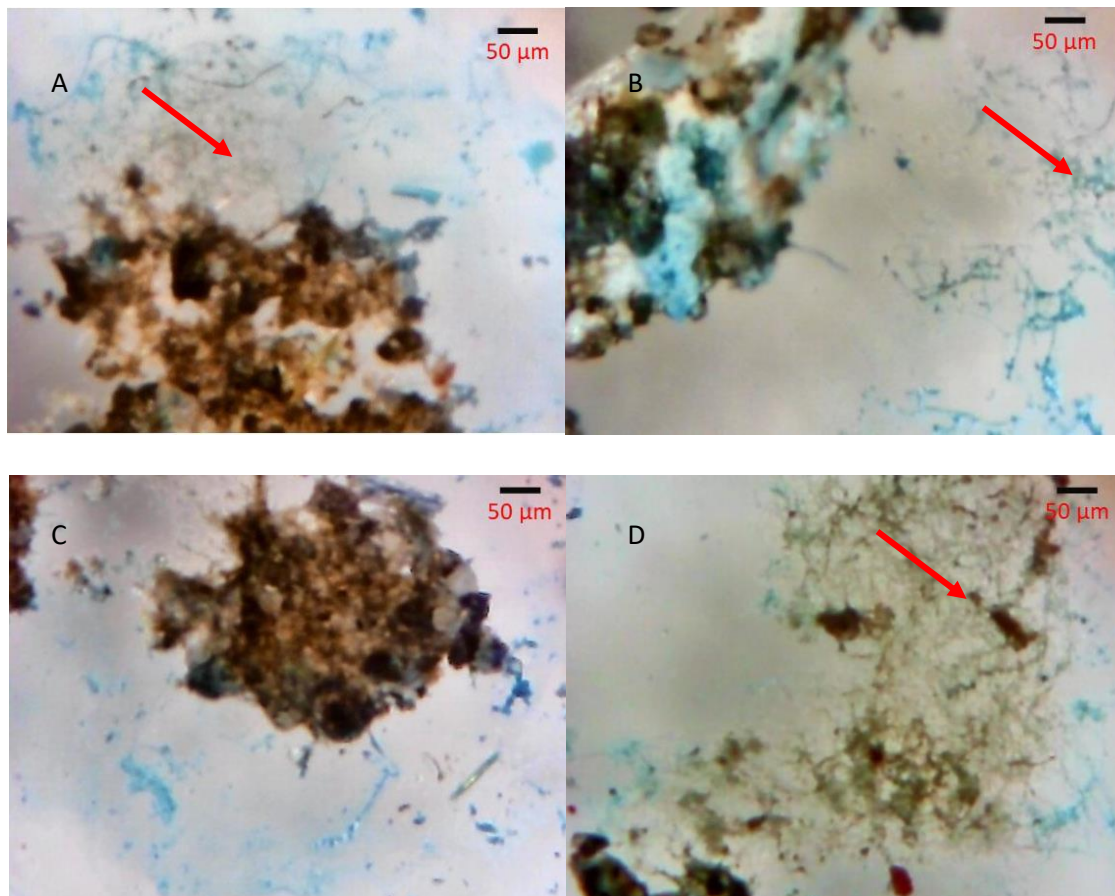


Figure 6. Microscopic appearance of scraped surfaces of the paving blocks after 14 days of subtidal immersion. A). 5%; B). 10%; C). 25% sponge extract *S. coralliphagum*.; D). Negative control. Note: Red arrows= fiber-like formation

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

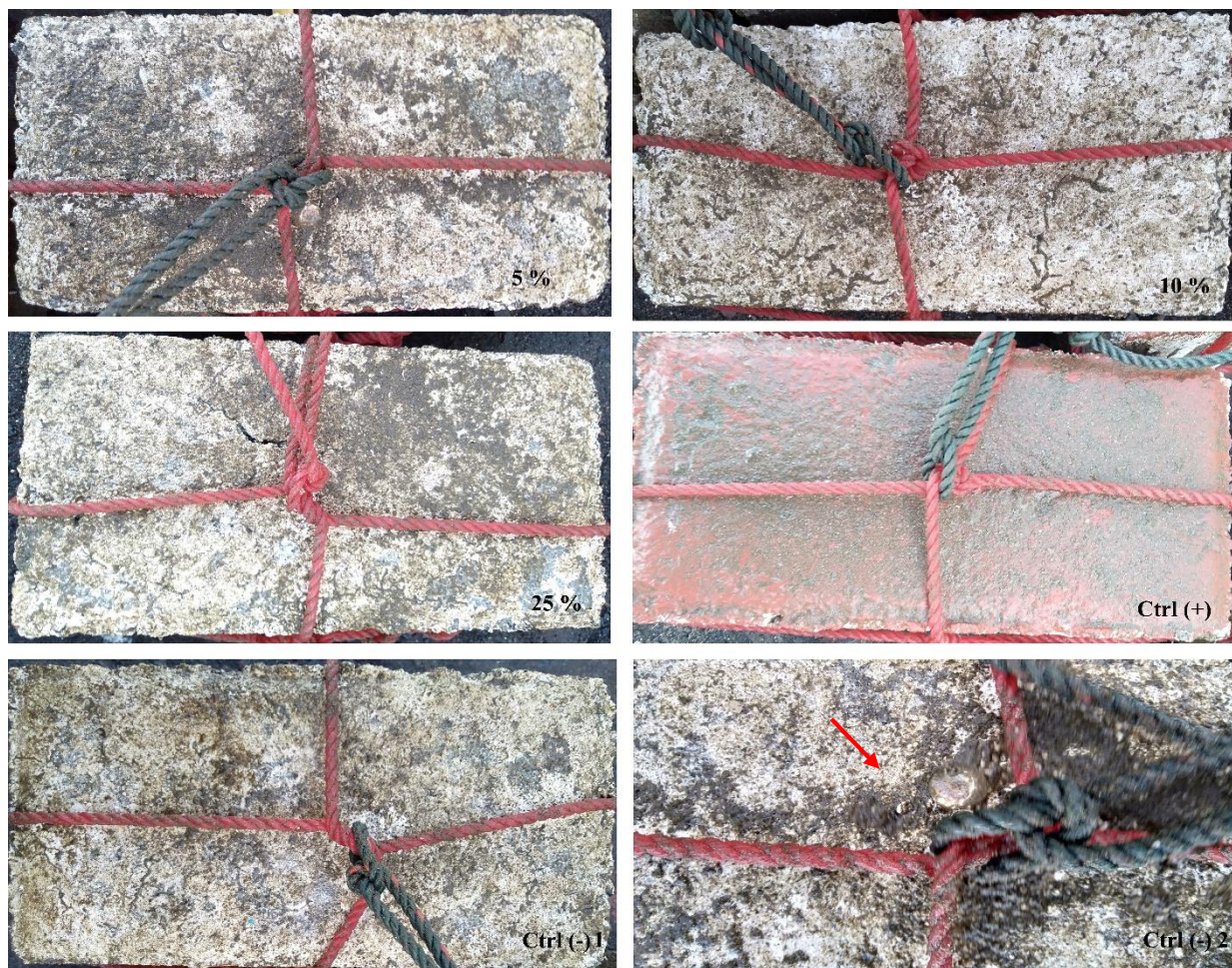


Figure 7. Display of paving blocks with different treatments after 21 days of subtidal immersion. Note: Red arrows = macro fouler tunicate *Corella eumyota*

The observations of the 21st day are presented in Figure 7. At 21 days after subtidal submergence, a macro fouler, i.e., a tunicate, *Corella eumyota*, was observed attached to the negative control. No macro fouler was observed on all treatments and positive control. Sediment deposition on the surface of paving blocks looks more than observed in the

previous 14th days. Microscopic observations show zooplankton colonization on the surfaces of the paving blocks (digital data in the form of moving pictures can be accessed at https://drive.google.com/file/d/1VyW8tmNhO_IBHBBfRbnxotJ1eDI89JnX/view?usp=sharing)

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*



Figure 8. Display of paving blocks with different treatments after 28 days of subtidal immersion.

The result of the 28th-day observation is presented in Figure 8. At 28 days after subtidal immersion, no macroscopic organisms were attached to all treatments and in both controls. The observed tunicate *Corella eumyota* which had previously begun to grow in the negative control was no longer kept (Figure 7); it was very likely to be predated by littoral fish or *Ophiolepis* sp., which was also found on the paving. Microscopic observations showed that some of the

thalli of green algae were seen on the paving surface in treatment 1 (5%) and in the negative control. The presence of the thalli indicates the settlement process of microalga; the environmental changes at the microscopic level of the paving surface are marked by the initial formation of a biofilm layer by bacteria that occurred on the 14th day to be most likely to provide nutrients that allow the process to take place [20].

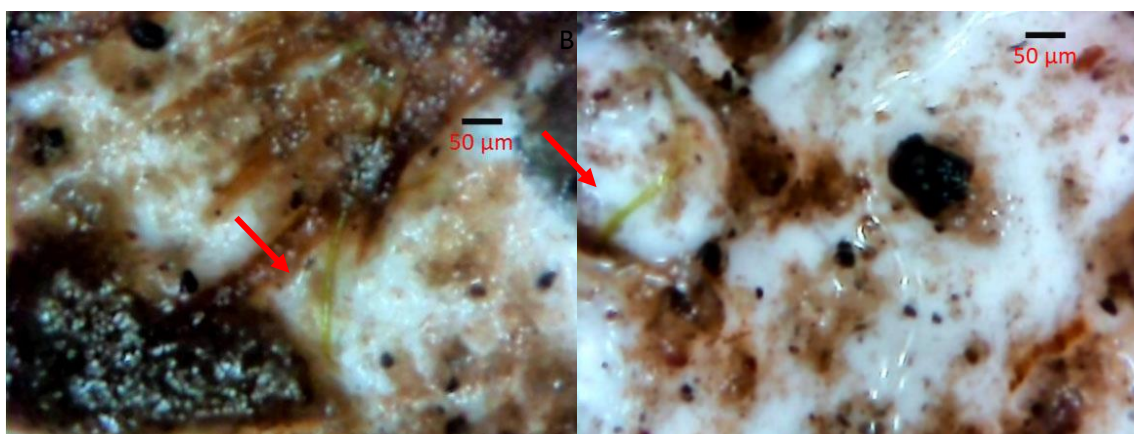


Figure 9. Microscopic appearance of the surfaces of paving block after 28 days of subtidal immersion, A) Negative control; B). 5% extract. Red arrows = thalli formation of green microalga.

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*



Figure 10. Display of paving blocks with different treatments after 90 days of subtidal immersion. Note Red arrows = macro-fouling organisms.

After the 90th day, it was observed that macro-foulers could only develop in the negative and positive control (Figure 10). The development of fouling organisms was not observed in all treatments 5, 10, or 25% extract of *S. coralliphagum*. Fouling organisms observed were juvenile gastropods

Pinctada margaritifera, *P. maxima*, and *Cerithidea rizophorarum*. From the sponge species, *Niphates erecta*, and the tunicate species, *Corella eumyota*. In contrast, the alga *Ulva lactuca* was observed only in negative control (Figure 10).



Figure 11. Microscopic appearance of the surface of negative control paving block after 90 days of subtidal immersion

Microscopic observations show the formation of a mycelium structure from the fungus *Fusarium cf. tricinctum* with fiber-like pink color; this structure forms a biofilm layer on top of organic material underneath it, which is very likely formed

from bacterial biofilm that grew previously from day 14 (Figure 11). We have also observed settled micro alga that have developed and are attached to the surface of the substrate (indicated by red arrows).

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

Tables 1 and 2 summarize the organisms found on the paving blocks with different treatments.

Table 1. List of fouling microorganisms on paving blocks treatments

Paving blocks Treatments	Days of subtidal immersion				
	7 days	14 days	21 days	28 days	90 days
5% w/v	-	Bacterial hyphae formation	-	Thallus of microalga	-
10 % w/v	-	Bacterial hyphae formation	-	-	-
25 % w/v	-	-	-	-	-
Positive Ctrl	-	-	-	-	-
Negative Ctrl	-	Bacterial hyphae formation	-	Microalga	Mycelium of <i>Fusarium cf. tricintum</i>

Table 2. List of fouling macroorganisms growing on paving blocks treatments

Paving blocks Treatments	Days of subtidal immersion				
	7 days	14 days	21 days	28 days	90 days
5% w/v	-	-	-	-	-
10 % w/v	-	-	-	-	-
25 % w/v	-	-	-	-	-
Positive Ctrl	-	-	-	-	Gastropod: <i>Pinctada margaritifera</i>
Negative Ctrl	-	-	Tunicate: <i>Corella eumyota</i>	-	Gastropod: <i>P. margaritifera</i> <i>P. maxima</i> <i>Cerithidea rizophorarum</i> Sponge: <i>Niphates erecta</i> Tunicate: <i>Corella eumyota</i> Green alga: <i>Ulva lactuca</i>

Antibacterial analysis

Eleven fractions of the *S. coralliphagum*'s extract were submitted to bioactivity test against Gram-negative prokaryote *Escherichia. coli* DSM 498, as shown in Table 3.

Based on the result, only DCM:EtoAc=3:7 fraction show activity against tested microorganisms. The polarity of the active compound of *S. coralliphagum* belongs to semi-polar. No other fractions detected no activity [24].

Table 3. Bioactivity test of *S. coralliphagum* fractions against bacteria

No.	Fractions of <i>S. coralliphagum</i> extract	Activity against	
		<i>B. megaterium</i> DSM 32 ^T	<i>E. coli</i> DSM 498
1	Petroleum Ether 100 %	-	-
2	Dichloromethane (DCM) 100%	-	-
3	DCM : Ethylacetate (EtoAc); 7:3	-	-
4	DCM : EtoAc ; 1:1	-	-
5	DCM : EtoAc ; 3:7	-	20.0±0.81 mm
6	EtoAc 100%	-	-
7	EtoAc : Methanol (MeOH) 7:3	-	-

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

8	EtOAc : MeOH; 1:1	-	-
9	EtOAc : MeOH; 3:7	-	-
10	Acetone 100%	-	-
11	MeOH 100%	-	-

NMR analysis

The active fraction of *S. coralliphagum* was then sent to ^1H NMR to analyze its chemical structure (Figure 12). In general, the active fraction still contains long fatty acid compounds. Furthermore, the active fraction might consist of a cyclic aliphatic compound with keto-enol functional groups

attached to the system, which was shown in 4.0-4.5 ppm, followed by several methyl aliphatic groups at the end of its chain (appears at 1.5 ppm) and has been observed based on ^1H NMR spectrum. Due to the less amount of the fraction, we are not able to elucidate the exact chemical structure of the active compound

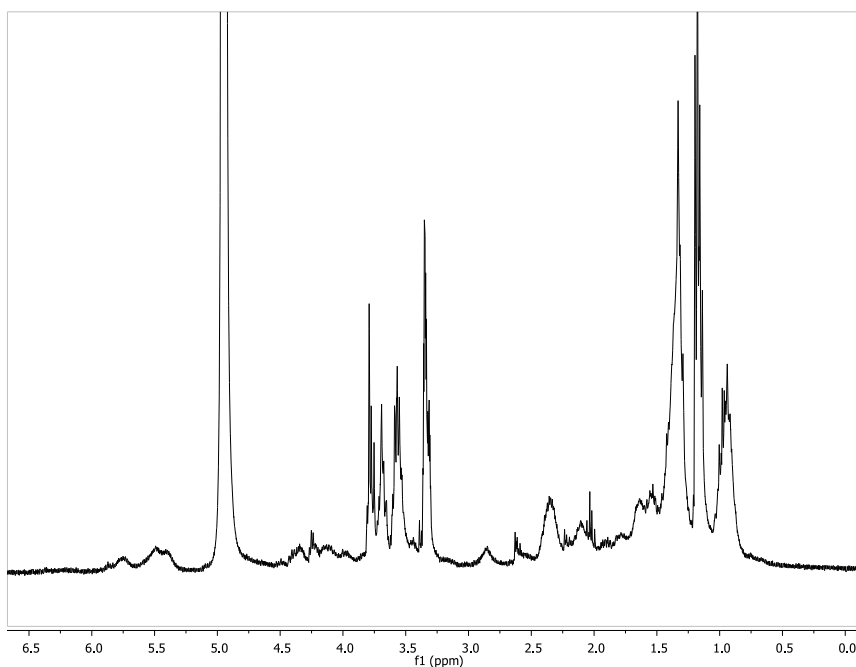


Figure 12. ^1H NMR spectrum of Active Fraction (DCM : EtOAc ; 3:7) of *S. coralliphagum*

Species *S. coralliphagum* has been long studied regarding their chemical importance for humanity. Several new metabolites derived from related species, e.g. siphonodictyal C-E, siphonodictyol G, H, and siphonodictyoic acid isolated from species growing in several places in the Pacific and Caribbean. All these compounds show activity against an array of clinical isolates, i.e., *Staphylococcus aureus*, *Bacillus subtilis*, and *Vibrio anguillarum* [25]. The particular sponge also consists of protein kinase C (PKC) inhibitors from the sesquiterpene group and corallidictyals A and B from a sponge that grows in the Little San Salvador Waters of Bahamas [24]. Selective inhibitors of PKC are presently being developed as potential therapies to fight cancer, inflammatory and cardiovascular diseases. Also, Killday *et al.* isolated unprecedented desulfated sesquiterpene-hydroquinone, bis(sulfato)-cyclosiphonodictyol A, which inhibit the binding of [^3H]-LTB₄ to intact human neutrophils which can be led as immunotherapies-related diseases. This deep-water sponge grew in the Bahamas [26].

CONCLUSIONS

The extract sponge *S. coralliphagum* has vigorous antifouling activity against marine fouling organisms. The extract was fractionated using VLC and continued by testing their activity against Gram-negative bacteria where DCM:EtOAc=3:7 fraction is active. Investigation of NMR spectra of Fr. DCM:EtOAc=3:7 shows a cyclic aliphatic compound with keto-enol functional groups attached to the system followed by several methyl aliphatic groups at the end of its chain.

Conflict of Interests: There are no conflicts of interest

REFERENCES

- I. P. Bhadury, B.T. Mohammad, P.C.J.J.o.I.M. Wright, and Biotechnology, *The current status of natural products from marine fungi and their potential as anti-infective agents*. **33**(5): p. 325. (2006)
- II. A. Bazes, A. Silkina, P. Douzenel, F. Faÿ, N. Kervarec, D. Morin, J.-P. Berge, and N.J.J.o.A.P. Bourgougnon, *Investigation of the antifouling constituents from the brown alga Sargassum*

Investigation of an Antifouling Compound from Sponge *Siphonodictyon coralliphagum*

- muticum* (Yendo) Fensholt. **21**(4): p. 395-403. (2009)
- III. M. Mbadanga, F. Schoefs, V. Quiniou-Ramus, M. Birades, and R. Garretta, *Marine Growth Colonization Process in Guinea Gulf: Data Analysis*. Journal of Offshore Mechanics and Arctic Engineering-Transactions of The ASME - J OFFSHORE MECH ARCTIC ENG. **129**(2007)
- IV. B.J. Baker, *Marine biomedicine from beach to bedside*. (2016)
- V. P. Proksch, *Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs*. Toxicon. **32**(6): p. 639-55. (1994)
- VI. P. Proksch, R. Edrada, and R. Ebel, *Drugs from the sea - current status and microbiological implications*. Applied Microbiology and Biotechnology. **59**(2): p. 125. (2002)
- VII. C. Jiménez, *Marine Natural Products in Medicinal Chemistry*. ACS Medicinal Chemistry Letters. **9**(10): p. 959-961. (2018)
- VIII. G.M. König, S. Kehraus, S.F. Seibert, A. Abdel-Lateff, and D. Müller, *Natural Products from Marine Organisms and Their Associated Microbes*. **7**(2): p. 229-238. (2006)
- IX. M. Assmann, R.W.M. van Soest, and M. Köck, *New Antifeedant Bromopyrrole Alkaloid from the Caribbean Sponge *Stylissa caribica**. Journal of Natural Products. **64**(10): p. 1345-1347. (2001)
- X. S.-T. Fang, B.-F. Yan, C.-Y. Yang, F.-P. Miao, and N.-Y. Ji, *Hymerhabdrin A, a novel diterpenoid with antifouling activity from the intertidal sponge *Hymerhabdia* sp.* The Journal Of Antibiotics. **70**: p. 1043. (2017)
- XI. B. Moon, B.J. Baker, and J.B. McClintock, *Purine and Nucleoside Metabolites from the Antarctic Sponge *Isodictya erinacea**. Journal of Natural Products. **61**(1): p. 116-118. (1998)
- XII. S.-H. Qi and X. Ma, *Antifouling Compounds from Marine Invertebrates*. **15**(9): p. 263. (2017)
- XIII. T. Turk, K. Sepčić, I. Mancini, and G. Guella, *3-Akylpyridinium and 3-Alkylpyridine Compounds from Marine Sponges, Their Synthesis, Biological Activities and Potential Use*, in *Studies in Natural Products Chemistry*, R. Atta ur, Editor. 2008, Elsevier. p. 355-397.
- XIV. R. Maradou, F. Losung, R. Mangindaan, R. Lintang, W. Pelle, and H. Sambali, *UJI AKTIVITAS ANTIBAKTERI BEBERAPA SPONS DARI PERAIRAN SALIBABU KEPULAUAN TALAUD*. JURNAL PESISIR DAN LAUT TROPIS. **7**: p. 234. (2019)
- XV. S.A. Pomponi, M.C. Diaz, R.W.M. Van Soest, L.J. Bell, L. Busutil, D.J. Gochfeld, M. Kelly, and M. Slattery, *Sponges*, in *Mesophotic Coral Ecosystems*, Y. Loya, K.A. Puglise, and T.C.L. Bridge, Editors. 2019, Springer International Publishing: Cham. p. 563-588.
- XVI. J. Moningka, *Uji Aktivitas antifouling beberapa jenis karang lunak*, in *Ilmu Kelautan* Sam Ratulangi University: Manado. (2000)
- XVII. Atta-ur-Rahman, M. Choudhary, and W. Thomsen, *Bioassay Techniques for Drug Development*, ed. Atta-ur-Rahman, M. Choudhary, and W. Thomsen. London: CRC Press. (2001)
- XVIII. F. Schoefs and M. Boukinda, *Modelling of marine growth effect on offshore structures loading using kinematics field of water particle*. (2004)
- XIX. C. Bressy and M. Lejars, *Marine Fouling : An Overview*. Journal of Ocean Technology. **9**: p. 19-28. (2014)
- XX. C. Richards, C. Briciu-Burghina, M.R. Jacobs, A. Barrett, and F. Regan, *Assessment of Antifouling Potential of Novel Transparent Sol Gel Coatings for Application in the Marine Environment*. **24**(16): p. 2983. (2019)
- XXI. D. Monroe, *Looking for chinks in the armor of bacterial biofilms*. PLoS biology. **5**(11): p. e307-e307. (2007)
- XXII. I.R. Alexander, *Marine Biofouling: Colonization Processes and Defenses*, CRC press. (2004)
- XXIII. M. Jamal, U. Tasneem, T. Hussain, S.J.F. Andleeb, R.i.H.I. Research, R.J.o. Microbiology, and Biotechnology, *Bacterial Biofilm: Its Composition*. **4**: p. 1-14. (2015)
- XXIV. J.A. Chan, A.J. Freyer, B.K. Carté, M.E. Hemling, G.A. Hofmann, M.R. Mattern, M.A. Mentzer, and J.W. Westley, *Protein Kinase C Inhibitors: Novel Spirosesquiterpene Aldehydes from a Marine Sponge Aka (=Siphonodictyon) coralliphagum*. Journal of Natural Products. **57**(11): p. 1543-1548. (1994)
- XXV. B.W. Sullivan, D.J. Faulkner, G.K. Matsumoto, C.H. He, and J. Clardy, *Metabolites of the burrowing sponge *Siphonodictyon coralliphagum**. The Journal of Organic Chemistry. **51**(24): p. 4568-4573. (1986)
- XXVI. K.B. Killday, A.E. Wright, R.H. Jackson, and M.A. Sills, *Bis(sulfato)-cyclosiphonodictyol A, a new disulfated sesquiterpene-hydroquinone from a deep water collection of the Marine sponge *Siphonodictyon coralliphagum**. J Nat Prod. **58**(6): p. 958-60. (1995)