Investigation of Antibiofilm Activities from Marine Sponges' Crude Extract of Pulau Bidong, Terengganu, Malaysia

Mohamad Gazali[2,3], *Wan* Nur Izzah Alissa Wan Rosli[1], *Siti* Sarah Mohd Zahidi[1], *Nur* Amira Jamaludin[2], *Kamariah* Bakar[2], and Jasnizat Saidin[1,2,*]

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia
[2]Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Malaysia
[3] Faculty of Fisheries and Marine Science, Teuku Umar University, Alue Peunyareng Street, Meureubo, West Aceh, 23681, Indonesia

Abstract. Biofilm, intricate microbial communities adhering to submerged surface, pose a significant challenge across various sectors. Conventional approaches relying on harsh chemical or mechanical removal have drawback. Marine organisms like sponges, with their diverse secondary metabolites, offer a promising avenue for developing sustainable solutions. This study investigated the antibiofilm potential of crude extracts from four marine sponges (*Stylissa carteri*, *Haliclona amboinensis*, *Hymeniacidon* sp., and *Xestospongia testudinaria*) against *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* biofilms. Crystal Violet staining quantified biofilm formation and removal. Well diffusion assays assessed antibacterial activity. *Haliclona amboinensis* and *Hymeniacidon* sp. extracts displayed antibiofilm effects against *V. parahaemolyticus* biofilm. The IC_{50} values for the activity were 0.1 mg/mL and 0.19 mg/mL, respectively. Notably, the *Hymeniacidon* sp. extract exhibited bactericidal activity whereas *H. amboinensis* lacked this effect. Interestingly, both extracts effectively removed pre-formed *V. parahaemolyticus* biofilms against *P. aeruginosa*, *H. amboinensis* displayed antibiofilm activity (IC_{50} = 2.8 mg/mL) without bactericidal effects. However, this extract did not impact pre-established *P. aeruginosa* biofilms. The study suggests *H. amboinensis* is a promising source for new antibiofilm agents. It effectively inhibits biofilm formation without killing bacteria. Further research is needed to identify the active compounds and their mechanisms for developing targeted therapies.

1 Introduction

Biofilms, composed primarily of proteins, nucleic acids, and polysaccharides within a polymeric matrix, are adherent microbial communities that colonize biotic and abiotic surfaces [1]. This mode of growth dominates various environments, including food

* Corresponding author: ijaxzt@umt.edu.my

processing, industrial settings, hospitals, and natural ecosystems [1]. Biofilms have been implicated in numerous outbreaks caused by pathogenic bacteria [2, 3]. Notably, over 80% of infections within the human body are attributed to biofilm-associated bacteria [4]. The significant challenge in biofilm eradication lies in their enhanced resistance (up to 1000-fold) to host immune responses and conventional antibiotics compared to their planktonic counterparts [5]. Therefore, exploring novel strategies to impede biofilm formation or eradicate established biofilms is of paramount importance.

Biofilm formation is a primary driver of biofouling, a major concern in aquaculture and is characterized by the attachment of diverse organisms to infrastructure and cultured organisms, consequently impacting productivity [6, 7]. Current strategies often rely on antifouling paints to manage the biofilm formation at the initial adhesion stage. However, these paints raise environmental concerns due to their hazardous nature [6, 8]. Identifying environmentally friendly antibiofilm agents is thus crucial for effective biofouling prevention. The marine environment harbors a vast treasure trove of novel bioactive compounds due to its remarkable biodiversity and chemical diversity [9]. Although bioactive compounds derived from marine microorganisms have been exploited for various applications, their potential in combating detrimental biofilms remains understudied [10]. This study aimed investigated the antibiofilm activity of marine sponges collected from Pulau Bidong against *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*.

2 Materials and methods

The experimental materials included marine sponges: *Stylissa carteri* (NCBI accession number MK473894; collection voucher number submitted to South China Sea Repository and Reference Centre, Universiti Malaysia Terengganu: UMT Spon 473894), *Hymeniacidon* sp. (MK473888; UMT Spon 473888), *Haliclona amboinensis* (MK43899; UMT Spon 43899), and *Xestospongia testudinaria* (MK903078; UMT Spon 903078). Chemicals used in this research include methanol (Merck), ethyl acetate (Merck), chloroform (Merck), n-hexane (Merck), DMSO (Merck), Crystal violet (Sigma Aldrich), *Pseudomonas aeruginosa* (ATCC 27853), *Vibrio parahaemolyticus* (ATCC 17802), Nutrient Agar (Oxoid), and Nutrient Broth (Merck). Instruments utilized in this research include a rotary evaporator Buchi R300, UV-Vis Spectroscopy (UV-800 Shimadzu), and an ELISA reader (SpectraMax® iD3 Multi-Mode Microplate Reader).

2.1 Sponge material

Sponge samples (Fig. 1) were collected in 2016 via scuba diving at depth of 5 to 20 meters at Pulau Bidong, Terengganu, Malaysia, located at a latitude of 103°03'42.6" E and a longitude of 5°36'16.7" N. Following collection, the samples were placed in an icebox and labeled. Subsequently, they were transported to the Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu, for further identification using a combination of traditional key taxonomy and DNA barcoding employing the cytochrome oxidase 1 gene (COI) [11]. Prior to the extraction procedure, the samples were thoroughly cleaned of any debris, ectobionts, and endobionts.

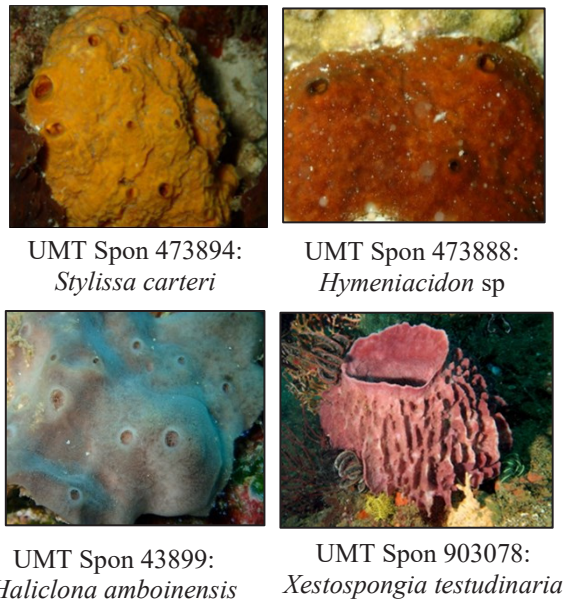

Fig. 1. The marine sponge used in this investigation captured at the time of sample gathering

2.2 Samples preparation and extraction

Following the removal of visible epiphytes with forceps, sponges were mechanically fragmented into small pieces using a scientific grinder. The fragmented material was freeze at -80°C and then lyophilized to remove water. The resulting dried samples (300-1000 mg) were subsequently macerated in 99.7% methanol for one week under constant agitation. After maceration, the solvent was evaporated using a rotary evaporator at 37°C, and yielding a crude residue [12-15]. The obtained crude extracts were weighed and stored at 4°C for further analysis. Prior to the biofilm assay, 15 mg of each extract was re-suspended in 200 µL of sterile nutrient agar (NA).

2.3 antibiofilm assay

2.3.1 Bacterial strains and culture preparation

Strains of *Pseudomonas aeruginosa* (ATCC 27853) and *Vibrio parahaemolyticus* (ATCC 17802) were obtained from the Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu. Cryopreserved bacterial stocks were revived on nutrient agar and marine agar plates, respectively, for optimal growth based on established protocols [16]. The plates were incubated for 24 hours at room temperature. Subsequently, one to two colonies were selected from each agar plate and used to inoculate separate flasks containing 5 mL of respective broth. These overnight cultures of *P. aeruginosa* and *V. parahaemolyticus* were then incubated for an additional 24 hours at 37°C with shaking to ensure homogeneous growth. The optical density (OD_{600}) of each culture was measured at a wavelength of 600 nm using a UV-Vis spectrophotometer (UV-800 Shimadzu). Cultures were then adjusted to an OD_{600} of 0.2 using sterile media for subsequent experiments.

2.3.2 Crystal violet biofilm assay

A microtiter plate assay was used to assess the effect of the sponge crude extracts on biofilm formation by *P. aeruginosa* and *V. parahaemolyticus*. Overnight cultures of each bacterial strain, adjusted to a 0.2 of OD_{600}, were used as inocula. Aliquots 100 µL of the inoculum were dispensed into each well of a sterile 96-well plate. Each well contained 100 µL of the

respective bacterial broth supplemented with varying concentrations of the sponge's crude extract. A two-fold dilution series, ranging from 7.5 to 0.23 mg/mL, was prepared to evaluate the dose-dependent effect of the extracts. The nutrient broth or marine broth without the extract as the negative control. Positive control wells included 0.025% (w/v) sodium hypochlorite (NaOCl) solution for *V. parahaemolyticus* and 0.16% for *P. aeruginosa* [17, 18] to represent a known antibiofilm agent. The plate was incubated at 37°C for 24 hours to allow biofilm formation. Following incubation, planktonic cells were removed, and adherent biofilm was quantified using a modified crystal violet staining method [19]. Briefly, the contents of each well were discarded, and the plate was dried at 60-70°C for 30 minutes in a drying oven. Subsequently, 200 µL of 0.1% (wt/vol) crystal violet solution was added to each well and allowed to stain for 5 minutes. The staining solution was then discarded, and the wells were gently rinsed with tap water until the rinse water ran clear. A second drying step at 60-70°C for 30 minutes followed. To quantify the adhered biofilm, 200 µL of 70% ethanol was added to each well to solubilize the crystal violet stain. The mixture was incubated at room temperature for 30 minutes. The absorbance of the crystal violet-stained biofilms in each well was measured at 595 nm using a microplate reader (SpectraMax® iD3 Multi-Mode Microplate Reader). The percentage of biofilm inhibition was calculated using the method described by Leroy [20]. The entire experiment was performed in triplicate for data reproducibility.

2.3.3 Evaluation of antibacterial activity

To distinguish between a bactericidal mode of action or other mechanisms underlying the extract's antibiofilm activity, a well-diffusion assay was performed on Mueller-Hinton agar for respective positive antibiofilm samples. This assay assesses the presence and diameter of the inhibition zones surrounding the extract, indicating its ability to directly suppress bacterial growth. Briefly, standardized inocula 0f 0.2 OD_{600} of either *P. aeruginosa* or *V. parahaemolyticus* (100 µL) were uniformly spread across the agar surface using a sterile spreader. Sterile well-makers (e.g., cork borers) were employed to create wells in the agar following the protocol described by Balouiri [21]. Fifty microliters of *H. amboinensis* and *Hymeniacidon* sp. crude extracts (approximately 3 to 7 times of IC_{50} of antibiofilm from previous experiments: 7.5 mg/mL for *H. amboinensis* against *P. aeruginosa* and 1.88 mg/mL for both extracts against *V. parahaemolyticus*) were dispensed into the wells. An oxytetracycline disc (30 µg) was used as the positive control. The plates were then incubated at 37°C for 24 hours. The diameters of the inhibition zones surrounding the wells and the positive control disc were measured using calibrated Vernier calipers or a metric ruler in millimeters (mm).

2.3.4 Biofilm removal assay

The ability of marine sponge crude extracts to remove pre-established biofilms was evaluated using a modified microtiter plate assay based on the method described by Song [22]. Briefly, 200 µL aliquots of bacterial cultures (OD_{600} of 0.2) were dispensed into each well of a sterile 96-well plate and incubated for 48 hours at 37°C to allow biofilm formation. After the incubation period, 100 µL of the culture medium was discarded.

One hundred microliters of *H. amboinensis* and *Hymeniacidon* sp. crude extracts (approximately 3 to 7 times of IC_{50} of antibiofilm from previous experiments: 7.5 mg/mL for *H. amboinensis* against *P. aeruginosa* and 1.88 mg/mL for both extracts against *V. parahaemolyticus*) were dispensed into the wells. Marine broth (for *V. parahaemolyticus*) or nutrient broth (for *P. aeruginosa*) served as negative controls. Sodium hypochlorite (NaOCl) solutions (0.025% for *V. parahaemolyticus* and 0.16% for *P. aeruginosa*) were used as positive controls. The plates were incubated for an additional 24 hours at 37°C to facilitate interactions between the extracts and pre-formed biofilms. Following incubation, the

planktonic cells were removed, and the remaining adherent biofilm was quantified using a crystal violet (CV) staining assay as described in the previous section.

2.4 Statistical analysis

The experiments were conducted in triplicate, and the mean values and standard deviations of the absorbance were expressed. The formula by Nadri [23] was used to calculate the percentage of biofilm inhibition (%). A plot was created by graphing the percentage of biofilm inhibition (%) versus the crude extract concentration (mg/mL). By means of linear interpolation, the crude extracts' IC_{50} was ascertained. The standard deviation of the mean (SEM) was used to present the data. GraphPad Prism (GraphPad Software, San Diego, CA, USA) version 8.0 was used to statistically analyse the data. The groups were compared using a one-way analysis of variance (ANOVA) and Turkey's post hoc test. Statistical significance was defined as a p-value of less than 0.05.

3 Result and Discussion

3.1 Antibiofilm activity of sponges crude extract

The extract of *H. amboinensis* demonstrated antibiofilm activity against *P. aeruginosa* and *V. parahaemolyticus* with IC_{50} value of 2.8 and 0.1 mg/mL, respectively. *Hymeniacidon* sp. only exhibited IC_{50} against *V. parahaemolyticus* with value of 0.19 mg/mL. None of the extracts from *Stylissa carteri* and *Xestospongia testudinaria*, showed biofilm inhibition activity on the both bacteria used (Fig. 2 and IC_{50} values were summarized in Table 1). Based on the results, *H. amboinensis* and *Hymeniacidon* sp. were subjected to further experimentation.

Table 1. Antibiofilm of marine Sponges crude extract against *P. aeruginosa* and *V. parahaemolyticus*

Marine Sponges	**Antibiofilm Activities (mg/mL)**	
	P. aeruginosa	***V. parahaemolyticus***
Haliclona amboinensis	2.8	0.10
Hymeniacidon sp.	No IC_{50}	0.19
Stylissa carteri	No IC_{50}	No IC_{50}
Xestospongia testudinaria	No IC_{50}	No IC_{50}

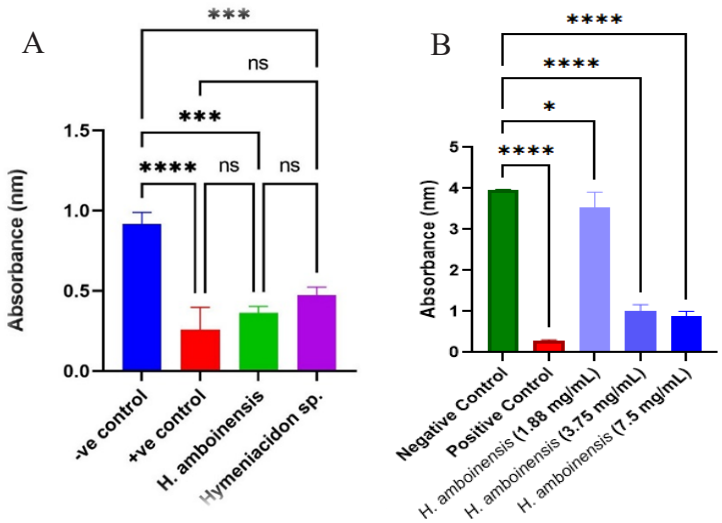

Fig. 2. A- Antibiofilm activity of *H. amboinensis* and *Hymeniacidon* sp. against *P. aeruginosa* biofilm; B- Antibiofilm activity of *H. amboinensis* against *V. parahaemolyticus* biofilm. Negative control consists of bacteria in respective broth, while positive control consists of v/v % NaOCl and bacteria in respective broth. The bars on the graph represent mean ± SD of biofilm formation from three replicates. The Tukey's Post Hoc test showed a statically significant different relative to control ($p < 0.001$).

One of the creative tactics that have been researched over the past 20 years to stop the formation of biofilms—which are produced by over 90% of bacteria—is the use of natural products [24, 25]. More than 30% of marine natural products come from marine sponges, which are also known for having antibiofilm modulators. This was demonstrated in the current investigation, which found that two of the four marine sponges evaluated have antibiofilm properties against *V. parahaemolyticus*. *Hymeniacidon sp.* and *H. amboinensis* are the two species of marine sponges. To our knowledge, no research has previously been done on the antibiofilm activity of *Hymeniacidon sp.* and *H. amboinensis* specifically against the biofilm of *V. parahaemolyticus*. However, research revealed that several chemicals, including pyrroloiminoquinone alkaloids and bromopyrrole, were present in *Haliclona* spp. and *Hymeniacidon* spp. [26, 27]. According to a study by Sun [28] one of the bromopyrrole alkaloids, oroidin, demonstrated the inhibitory effects against a panel of Gram-negative bacteria, proving that among these two compounds, bromopyrrole compounds can inhibit biofilm. Research by Kelly [29] provides evidence in support of their conclusions. Furthermore, it has been discovered that oroidin strongly prevents the growth of bacteria, both Gram-positive and Gram-negative [30].

3.2 Evaluation of antibacterial activity

Of the two marine sponges, only *Hymeniacidon* sp. exhibited antibacterial activity with inhibition zone against *V. parahaemolyticus*. This was evident in the formation of an inhibition zone with the diameter of 17 mm, while *H. amboinensis* showed no antibacterial activity on *P. aeruginosa* and *V. parahaemolyticus.*

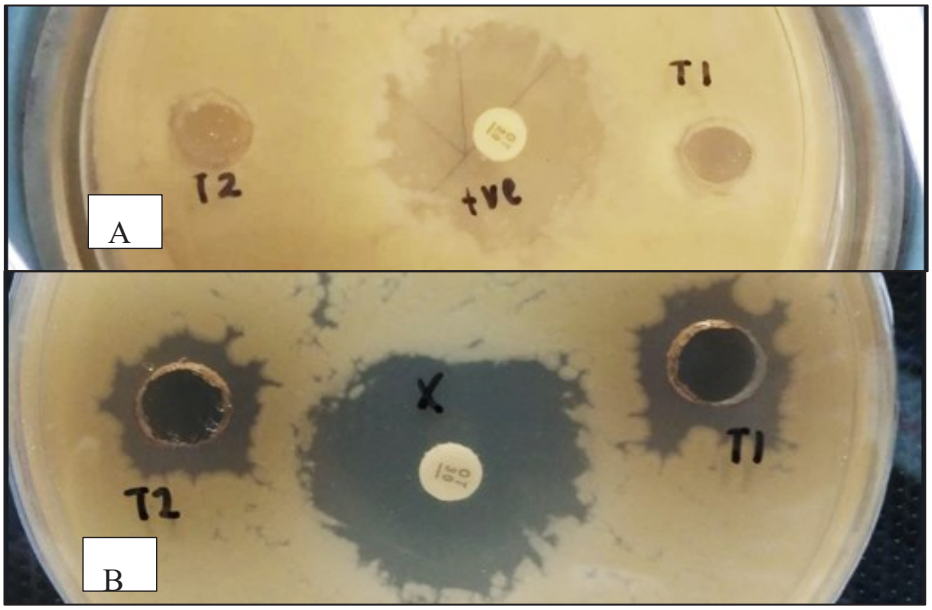

Fig. 3. The inhibitory zone diameter of 1.88 mg/mL of (A) *H. amboinensis* and (B) *Hymeniacidon* sp. against *V. parahaemolyticus*. Oxytetracycline disc (30 µg) served as the positive control

The inhibition zone indicates that *Hymeniacidon* sp. produces substances that can prevent or inhibit the growth of *V. parahaemolyticus* bacteria. In contrast, *H. amboinensis* exhibited no antibacterial effects against either *P. aeruginosa* or *V. parahaemolyticus*. This suggests that *H. amboinensis* may contain different bioactive compounds or may not produce compounds that are effective against these particular bacteria.

3.3 Biofilm removal assay

There was no significant difference of matured *P. aeruginosa* biofilm removal by *H. amboinensis* observed (Figure 3A). As for matured *V. parahaemolyticus* biofilm removal by *H. amboinensis* and *Hymeniacidon* sp. there were some weak removal activities were observed (Fig. 3B).

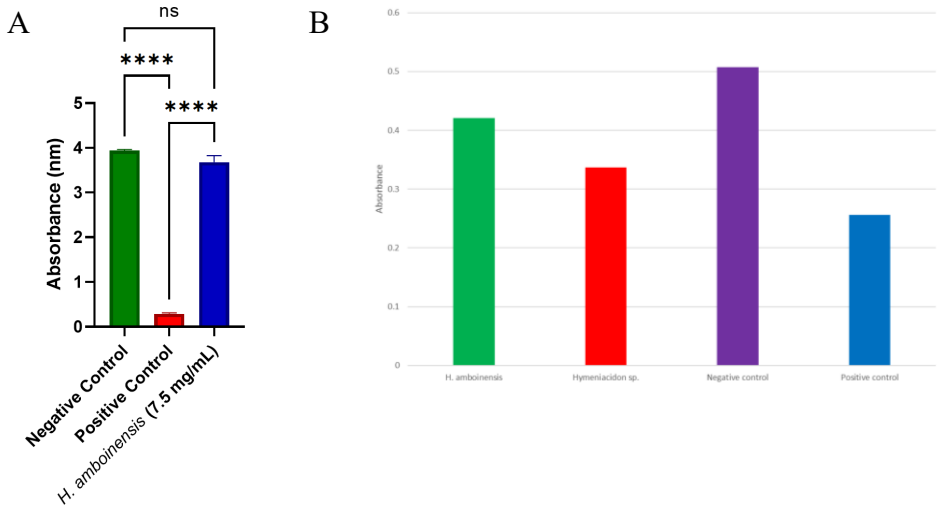

Fig. 4. A- Matured *P. aeruginosa* biofilm removal by *H. amboinensis*. B- matured *V. parahaemolyticus* biofilm removal by *H. amboinensis* and *Hymeniacidon* sp. The Tukey's Post Hoc test showed a statically significant different relative to control ($p < 0.001$). ns-not significant difference.

As seen in Fig. 4, where both sponges were able to remove matured biofilm by *V. parahaemolyticus*, all of these reports may also help to confirm biofilm removal activity. Apart from *H. amboinensis*'s capacity to eliminate mature biofilm produced by *V. parahaemolyticus*, Khotimchenko [31] have reported that specific diterpenes obtained from the sponge *Haliclona* sp., namely haliclonadiamine (a quorum sensing inhibitor), have exhibited the capability to obstruct the generation or interpretation of autoinducers. It is thought that *X. testudinaria* and *S. carteri* have weak antibiofilm action. Our results contradict those of earlier research that found *S. carteri* and *X. testudinaria* to have strong antibiofilm action [32, 33]. This could be the result of biological differences between the sites where the marine sponges were collected; hence, environmental changes could produce different secondary metabolites and alter the chemical makeup of the sponges [34, 35]. As a result, their antibiofilm actions differ from our discoveries as well.

4. Conclusion

Both *H. amboinensis* and *Hymeniacidon* sp. demonstrated promising anti-biofilm activity against *P. aeruginosa* and *V. parahaemolyticus*. While *Hymeniacidon* sp. exhibited bactericidal properties against *V. parahaemolyticus* thus preventing biofilm formation. *Haliclona amboinensis* exhibited a non-bactericidal mechanism, suggesting it as a potential alternative for combating biofilms without toxicity toward bacterial communities and also toward marine environment.

References

1. H.C. Flemming, J. Wingender, The biofilm matrix. Nature Reviews Microbiology **8**(9), 623–633 (2010). https://doi.org/10.1038/nrmicro2415
2. Lapidot, U. Romling, S. Yaron, Biofilm formation and the survival of Salmonella Typhimurium on parsley. International Journal of Food Microbiology, **109**(3), 229–233 (2006). https://doi.org/10.1016/J.IJFOODMICRO.2006.01.012
3. K. Aarnisalo, J. Lundén, H. Korkeala, G. Wirtanen, Susceptibility of Listeria monocytogenes strains to disinfectants and chlorinated alkaline cleaners at cold

temperatures. LWT-Food Science and Technology, **40**(6), 1041–1048 (2007). https://doi.org/10.1016/J.LWT.2006.07.009
4. S. Moreau-Marquis, B.A. Stanton, G.A. O'Toole, Pseudomonas aeruginosa biofilm formation in the cystic fibrosis airway. Pulmonary Pharmacology & Therapeutics, **21**(4), 595–599 (2008). https://doi.org/10.1016/J.PUPT.2007.12.001
5. M. Burmølle, T. R. Thomsen, M. Fazli, I. Dige, L. Christensen, P. Homøe, M. Tvede, B. Nyvad, T. Tolker-Nielsen, M. Givskov, C. Moser, K. Kirketerp-Møller, H.K. Johansen, N. Høiby, P.Ø. Jensen, S.J. Sørensen, T. Bjarnsholt, Biofilms in chronic infections – a matter of opportunity – monospecies biofilms in multispecies infections. FEMS Immunology & Medical Microbiology, **59**(3), 324–336 (2010). https://doi.org/10.1111/J.1574-695X.2010.00714.X
6. K.A. Dafforn, Lewis, J.A., Johnston, E.L. Antifouling strategies: History and regulation, ecological impacts and mitigation. Marine Pollution Bulletin, **62**(3), 453–465 (2011). https://doi.org/10.1016/J.MARPOLBUL.2011.01.012
7. I. Fitridge, T. Dempster, J. Guenther, R. de Nys, The impact and control of biofouling in marine aquaculture: A review. *Biofouling* **28**, 649–669 (2012). https://doi.org/10.1080/08927014.2012.700478
8. S. Wu, G. Liu, D. Zhang, C. Li, C. Sun, Purification and biochemical characterization of an alkaline protease from marine bacteria *Pseudoalteromonas* sp. 129-1. J. Basic Microbiol. 55, 1427–1434 (2015). https://doi.org/10.1002/JOBM.201500327
9. B.K. Carte, Marine natural products as a source of novel pharmacological agents. Curr. Opin. Biotechnol. **4**, 275–279 (1993). https://doi.org/10.1016/0958-1669(93)90095-E
10. L. Miao, P.Y. Qian, Antagonistic antimicrobial activity of marine fungi and bacteria isolated from marine biofilm and seawaters of Hong Kong. Aquat. Microb. Ecol. **38**, 231–238 (2005). https://doi.org/10.3354/AME038231
11. Bakar, N.A. Jamaludin, J. Saidin, Checklist of marine sponges off Bidong Archipelagos based on COI gene sequences. J. Sustain. Sci. Manag. **19**, 12-19 (2024). http://doi.org/10.46754/jssm.2024.01.002
12. V. Rao, V. Rao, Phytochemicals - A global perspective of their role in nutrition and health. Phytochemicals - A Global Perspective of Their Role in Nutrition and Health (2012). https://doi.org/10.5772/1387
13. Z.B. Ujang, T. Subramaniam, M.M. Diah, H.B. Wahid, B.B. Abdullah, A.H. Bin, A. Rashid, D. Appleton, Bioguided fractionation and purification of natural bioactives obtained from *Alpinia conchigera* water extract with melanin inhibition activity. J. Biomater. Nanobiotechnol. 265–272 (2013). https://doi.org/10.4236/JBNB.2013.43033
14. Pandey, S. Tripathi, Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. **2**, 115–119 (2014)
15. A.R. Abubakar, M. Haque, Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J. Pharmacy Bioallied Sci. 12, 1 (2020). https://doi.org/10.4103/JPBS.JPBS_175_19
16. N.A. Jamaludin, K. Bakar, J. Saidin, In vitro biological activity of three marine sponges from Theonella and Haliclona genera collected from Bidong Island, Terengganu, Malaysia. Malaysian Appl. Biol. **52**, 51–59 (2023). https://doi.org/10.55230/MABJOURNAL.V52I2.2559
17. C. Estrela, R.G. Ribeiro, C.R.A. Estrela, J.D. Pécora, M.D. Sousa-Neto, Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz. Dent. J. 14, 58–62 (2003). https://doi.org/10.1590/S0103-64402003000100011

18. C.B. Lineback, C.A. Nkemngong, S.T. Wu, X. Li, P.J. Teska, H.F. Oliver, Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds. Antimicrob. Resist. Infect. Control **7**, 1–7 (2018). https://doi.org/10.1186/S13756-018-0447-5/FIGURES/2
19. E.F. Haney, M.J. Trimble, R.E.W. Hancock, Microtiter plate assays to assess antibiofilm activity against bacteria. Nat. Protoc. **16**, 2615–2632 (2021). https://doi.org/10.1038/s41596-021-00515-3
20. C. Leroy, C. Delbarre-Ladrat, F. Ghillebaert, M.J. Rochet, C. Compère, D. Combes, A marine bacterial adhesion microplate test using the DAPI fluorescent dye: A new method to screen antifouling agents. Lett. Appl. Microbiol. **44**, 372–378 (2007). https://doi.org/10.1111/J.1472-765X.2006.02103.X
21. M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal. **6**, 71–79 (2016). https://doi.org/10.1016/J.JPHA.2015.11.005
22. X. Song, Y. Ma, J. Fu, A. Zhao, Z. Guo, P.K. Malakar, Y. Pan, Y. Zhao, Effect of temperature on pathogenic and non-pathogenic Vibrio parahaemolyticus biofilm formation. Food Control **73**, 485–491 (2017). https://doi.org/10.1016/J.FOODCONT.2016.08.041
23. M.H. Nadri, Y. Salim, N. Basar, A. Yahya, R.M. Zulkifli, Antioxidant activities and tyrosinase inhibition effects of Phaleria macrocarpa extracts. Afr. J. Tradit. Complement. Altern. Med. **11**, 107–111 (2014). https://doi.org/10.4314/AJTCAM.V11I3.16
24. B. Tan, J. Vanitha, Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: A review. Curr. Med. Chem. **11**, 1423–1430 (2004). https://doi.org/10.2174/0929867043365161
25. Lu, W. Hu, Z. Tian, D. Yuan, G. Yi, Y. Zhou, Q. Cheng, J. Zhu, M. Li, Developing natural products as potential anti-biofilm agents. Chinese Med. **14**, 1–17 (2019). https://doi.org/10.1186/S13020-019-0232-2/TABLES/3
26. E. Dogan, O. Demir, M. Sertdemir, M.A. Saracli, B. Konuklugil, Screening of selected marine sponges from the coasts of Turkey for antimicrobial activity. IJMS 47, 1193–1198 (2018). http://nopr.niscpr.res.in/handle/123456789/44482
27. K.S. Singh, M.S. Majik, Pyrrole-derived alkaloids of marine sponges and their biological properties. Stud. Nat. Prod. Chem. **62**, 377–409 (2019). https://doi.org/10.1016/B978-0-444-64185-4.00010-1
28. J. Sun, J. Wu, B. An, N.J. De Voogd, W. Cheng, W. Lin, Bromopyrrole alkaloids with inhibitory effects against the biofilm formation of Gram-negative bacteria. Marine Drugs **16**, 1 (2018). https://doi.org/10.3390/MD16010009
29. S.R. Kelly, P.R. Jensen, T.P. Henkel, W. Fenical, J.R. Pawlik, Effects of Caribbean sponge extracts on bacterial attachment. Aquat. Microb. Ecol. **31**, 175–182 (2003).
30. H. Zhang, Z. Khalil, M.M. Conte, F. Plisson, R.J. Capon, A search for kinase inhibitors and antibacterial agents: Bromopyrrolo-2-aminoimidazoles from a deep-water Great Australian Bight sponge, Axinella sp. Tetrahedron Lett. **53**, 3784–3787 (2012)
31. R. Khotimchenko, I. Bryukhovetskiy, M. Khotimchenko, Y. Khotimchenko, Bioactive compounds with antiglioma activity from marine species. Biomedicines **9**, 886 (2021).
32. J. Govinden-Soulange, D. Marie, S. Kauroo, R. Beesoo, A. Ramanjooloo, Antibacterial properties of marine sponges from Mauritius waters. Trop. J. Pharm. Res. **13**, 249–254 (2014).

33. Y.P. Cita, A. Suhermanto, O.K. Radjasa, P. Sudharmono, Antibacterial activity of marine bacteria isolated from sponge Xestospongia testudinaria from Sorong, Papua. Asian Pac. J. Trop. Biomed. **7**, 450–454 (2017).
34. N. Alves Junior, P.M. Meirelles, E. de Oliveira Santos, B. Dutilh, G.G.Z. Silva, R. Paranhos, A.S. Cabral, C. Rezende, T. Iida, R.L. de Moura, Microbial community diversity and physical–chemical features of the Southwestern Atlantic Ocean. Arch. Microbiol. **197**, 165–179 (2015).
35. D. Batista, R. Costa, A.P. Carvalho, W.R. Batista, C.P.J. Rua, L. de Oliveira, L. Leomil, A.M. Fróes, F.L. Thompson, R. Coutinho, Environmental conditions affect activity and associated microorganisms of marine sponges. Mar. Environ. Res. **142**, 59–68 (2018).