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BIOACTIVITIES OF THE MARINE SPONGE, *Stylissa carteri* AND CHARACTERIZATION OF ITS ACTIVE ANTIFOULING COMPOUND

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Biofouling causes huge economic losses and reduces facilities performances in oil and gas, and mariculture industries. Natural-based antifouling paint is needed to substitute high cost, less effective and toxic antifouling approaches. Despite being sessile, sponges are free from foulers and some species inhabiting Malaysian seawater remain unexplored for antifouling including *Stylissa carteri*. The objectives of this study were to determine the antibiofilm and antibacterial activities of methanolic crude extract (MCE) and fractions from marine sponge, *S. carteri* and to investigate the antibiofilm and antifouling activities of MCE-incorporated paint *in-vitro* and *in-situ*, respectively. We also characterized the compound involved in antifouling properties using spectroscopic techniques. *S. carteri* was macerated to produce the MCE and tested for antibiofilm activity against biofilm-producing bacteria, *Pseudomonas aeruginosa* using crystal violet biofilm assay. Disc-diffusion test was conducted to screen the antibacterial activity of the MCE against Gram-positive and negative bacteria. The MCE was incorporated into a paint with two different concentrations, 5% and 10% (w/v) and applied on steel panels. The coated panels were confirmed for antibiofilm activity in aquarium with seawater mimicking condition (*in-vitro*) for 24-hours while tested for antifouling activity in field (*in-situ*) for five months. The MCE was fractionated using Vacuum Liquid Chromatography and the fractions were tested

again for antibiofilm and antibacterial activities. Compound F4-4 was isolated from antibiofilm-active fraction, ScFP4 through trituration technique and characterized using Thin Layer Chromatography, Fourier Transform Infrared Spectroscopy, Liquid Chromatography-Mass Spectrometry, and Nuclear Magnetic Resonance Spectroscopy. The MCE showed promising results for antibiofilm with half-maximal inhibitory concentration (IC_{50}) of 20.22 µg/mL and produced a broad-spectrum antibacterial activity. Both concentrations of MCE-incorporated paint successfully reduced the biofilm formation tested *in-vitro* and prevented the settlement of barnacles *in-situ* as compared to blank paint. Fraction ScFP4 reduced the biofilm with IC_{50} of 2.40 µg/mL but exhibited no antibacterial activity indicating the independence of antibiofilm from antibacterial properties. According to spectroscopic data, compound F4-4 was identified as debromohymenialdisine (DBH, $C_{11}H_{11}N_5O_2$). In conclusion, *S. carteri* showed very promising antifouling performance in the presence of DBH which could serve as source of natural antifouling agents.

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BIOAKTIVITI SPAN MARIN, *Stylissa carteri* DAN PENCIRIAN KOMPAUN AKTIF ANTI-PENEMPELAN

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Bio-penempelan menyebabkan kerugian besar ekonomi dan mengurangkan prestasi fasiliti dalam industri minyak dan gas, dan marikultur. Cat anti-penempelan berasaskan bahan semulajadi diperlukan untuk menggantikan kaedah anti-penempelan yang tinggi kos, kurang berkesan dan toksik. Meskipun sesil, span bebas daripada penempel dan sesetengah spesies yang mendiami lautan Malaysia kekal tidak diterokai untuk anti-penempelan termasuklah *Stylissa carteri*. Objektif kajian ini adalah untuk menentukan aktiviti anti-biofilem dan anti-bakteria ekstrak mentah metanol (MCE) dan fraksinasi span marin, *S. carteri* dan untuk menyiasat aktiviti anti-biofilem dan anti-penempelan dalam cat yang mengandungi MCE masing-masing secara *in-vitro* dan *in-situ*. Kita juga mencirikan kompaun terlibat dengan sifat anti-penempelan menggunakan teknik spektroskopi. *S. carteri* direndam untuk mendapatkan MCE dan diuji untuk aktiviti anti-biofilem terhadap bakteria pembentuk biofilem, *Pseudomonas aeruginosa* menggunakan asai kristal ungu. Ujian resapan cakera juga dibuat untuk menyaring aktiviti anti-bakteria MCE terhadap bakteria Gram-positif dan negatif. MCE dimasukkan ke dalam cat dengan dua kepekatan berbeza, 5% dan 10% (w/v) dan diaplikasikan pada panel keluli. Panel bersalut dipastikan bagi aktiviti anti-biofilem dalam akuarium dengan kondisi menyerupai air laut (*in-vitro*) untuk 24-jam manakala diuji bagi anti-penempelan di lapangan (*in-situ*) untuk lima bulan. MCE

difraksinasikan menggunakan Kromatografi Cecair Bervakum dan fraksinasi diuji lagi bagi aktiviti anti-biofilem dan anti-bakteria. Kompaun F4-4 diasingkan daripada fraksinasi yang aktif anti-biofilem, ScFP4 melalui teknik traturasi dan dicirikan menggunakan Kromatografi Lapisan Nipis, Spektroskopi Inframerah Fourier Transformasi, Kromatografi Cecair-Spektrometri Jisim, dan Spektroskopi Resonans Magnetic Nuklear. MCE menunjukkan hasil yang menjanjikan bagi anti-biofilem dengan kepekatan perencatan separa maksima (IC_{50}) 20.22 µg/mL dan menghasilkan aktiviti anti-bakteria dengan spektrum yang luas. Kedua-dua kepekatan cat yang dimasukkan dengan MCE berjaya mengurangkan pembentukan biofilem yang diuji secara *in-vitro* dan menghalang penempelan teritip *in-situ* berbanding cat kosong. Fraksinasi ScFP4 mengurangkan biofilem dengan IC_{50} 2.40 µg/mL tetapi memaparkan tiada aktiviti anti-bakteria yang menunjukkan ketidakbergantungan ciri anti-biofilem pada anti-bakteria. Mengikut data spektrokopi, kompaun F4-4 telah dikenalpasti sebagai debromohymenialdisine (DBH, $C_{11}H_{11}N_5O_2$). Kesimpulannya, *S. carteri* menunjukkan pencapaian anti-penempelan yang sangat menjanjikan dengan kehadiran DBH yang boleh bertindak sebagai sumber ejen anti-penempelan semulajadi.