POTENTIAL ANTI-ATHEROSCLEROTIC COMPOUND FROM *Xestospongia* sp. OF BIDONG ARCHIPELAGO, TERENGGANU

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To..
Allah SWT for His blessing and love…
Ayah, Mak and family for the undying love and supports..
Lecturers and teachers for the guidance..
Friends for making life meaningful and enjoyable..

And wish not for the things in which Allah has made some of you to excel others. For men there is reward for what they have earned, (and likewise) for women there is reward for what they have earned, and ask Allah of His Bounty. Surely, Allah is Ever All-Knower of everything.

(An-Nisa': 32)

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

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Faculty : Science and Technology

Atherosclerosis or hardening of the arteries which occur due to gradual deposition of lipid, fibrin and calcium in the wall of arteries is one of the diseases with a high demand of new remedy. Marine organisms have been proven to contain bioactive compounds that possess many biological activities that are beneficial to humans. This study was carried out to isolate and characterize the potential bioactive compound present in marine sponge *Xestospongia* sp. and to determine the potential of bioactive compound isolated from *Xestospongia* sp. as an anti-atherosclerotic agent. Twenty eight specimens were collected, and sample selections were done based on cytotoxicity of the methanolic crude extract (MCE) samples towards human hepatocellular carcinoma liver cell line (HepG2) where 50% are toxic, 42.86% are non-toxic and 7.14% with undetermined cytotoxicity. *Xestospongia* sp. have been chosen to be partitioned into diethyl ether (DEF) and butanol (BEF) fractions, isolated and furthered with determination of potential anti-atherosclerotic activity that the sponge possesses. Isolation of the DEF of *Xestospongia* sp. yields a sterol compound SPO 001- DEF-KRF (1) based on TLC, IR, ^{1}H-NMR and ^{13}C-NMR spectrums . MCE, DEF, BEF and sterol compound SPO 001- DEF-KRF (1) of *Xestospongia* sp. were

treated onto HepG2 cells transfected with pGL3-PPRE in various concentrations ranging from 0.78 to 50.00 µg/ml and Rosiglitazone was used as positive control in the experiment. The MCE, DEF, BEF and sterol compound SPO 001- DEF-KRF (1) showed positive results to luciferase activities by increasing the transcriptional regulation of pGL3-PPRE at effective concentrations of 6.25, 12.5, 50.0 and 6.25 µg/ml respectively. Luciferase activity assay against SR-B1 promoter were conducted to confirm the ability of SPO 001-DEF-KRF (1) as a possible anti-atherosclerotic agent and Liver Receptor Homolog-1 (LRH-1) was used as positive control. Concentrations 6.25 µg/ml and 12.5 µg/ml were the most effective concentrations that increased the transcriptional regulation of the SR-B1 promoter by 1.9 fold when compared to negative control and was higher compared to the LRH-1. The result shows that the sterol SPO 001-DEF-KRF (1) exhibits a potential activity in increasing the transcriptional regulation of SR-B1 promoter which will subsequently increase the efficiency of the Reverse Cholesterol Transport (RCT) and finally lowering the risk of atherosclerosis in the body. These promising results show that *Xestospongia* sp. have the potential to be used as candidates for anti-atherosclerotic agent.

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SEBATIAN BERPOTENSI UNTUK ANTI-ARTERIOSKLEROTIK DARIPADA *Xestospongia* sp. DI KEPULAUAN BIDONG, TERENGGANU

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Arteriosklerosis atau pengerasan arteri yang berlaku akibat pemendapan secara beransur-ansur fibrin, lemak dan kalsium pada dinding arteri merupakan salah satu penyakit yang mempunyai permintaan tinggi terhadap ubat-ubatan baru. Organisma marin telah terbukti mengandungi sebatian bioaktif yang mempunyai aktiviti biologi yang bermanfaat kepada manusia. Kajian ini dijalankan bagi memencilkan dan mencirikan sebatian bioaktif yang berpotensi daripada span *Xestospongia* sp. dan untuk menentukan potensi sebatian bioaktif yang terpencil dari *Xestospongia* sp. sebagai agen anti-arteriosklerotik. Sebanyak 28 sampel telah dikumpulkan, dan pemilihan sampel dilakukan berdasarkan kesitotoksikan ekstrak metanol sampel (MCE) terhadap titisan sel hati hepatosellular karsinoma manusia (HepG2) di mana 50% adalah toksik, 42.86% tidak toksik dan 7.14% dengan kesitotoksikan yang tidak dapat ditentukan. *Xestospongia* sp. telah dipilih untuk difraksikan kepada fraksi dietil eter (DEF) dan Butanol (BEF), dipencilkan dan penentuan aktiviti anti- arteriosklerotik yang dimiliki oleh span tersebut telah dilakukan. Pemencilan fraksi DEF *Xestospongia* sp. menghasilkan sebatian Sterol SPO 001 - DEF-KRF (1) setelah diuji menggunakan TLC, IR, ^{1}H-NMR dan spektrum ^{13}C-NMR. Kesemua MCE,

DEF, BEF dan sebatian sterol SPO 001-DEF-KRF (1) *Xestospongia* sp. telah diuji ke atas sel HepG2 yang dijangkitkan dengan pGL3-PPRE dalam pelbagai kepekatan pada julat 0.78 sehingga 50.00 µg/ml dan Rosiglitazone telah digunakan sebagai kawalan positif di dalam eksperimen. MCE, DEF, BEF dan sebatian sterol SPO 001-DEF-KRF (1) menunjukkan hasil yang positif terhadap aktiviti luciferase dengan meningkatkan ungkapan transkripsi pGL3-PPRE pada kepekatan efektif masing-masing iaitu 6.25, 12.5, 50.0 dan 6.25 µg/ml. Pengasaian aktiviti luciferase terhadap promoter SR-B1 telah dijalankan untuk mengesahkan keupayaan SPO 001-DEF-KRF (1) sebagai ejen yang berpotensi untuk anti- arteriosklerotik dan reseptor hati homolog-1 (LRH-1) telah digunakan sebagai kawalan positif. Kepekatan 6.25 µg/ml dan 12.5 µg/ml didapati paling berkesan dalam meningkatkan ungkapan transkripsi promoter SR-B1 sebanyak 1.9 kali ganda apabila dibandingkan dengan kawalan negatif dan adalah lebih tinggi berbanding LRH-1. Hasil kajian menunjukkan bahawa sebatian sterol SPO 001-DEF-KRF(1) mempunyai potensi dalam meningkatkan ungkapan promoter SR-B1 yang kemudiannya akan meningkatkan kecekapan pengangkutan berbalik kolesterol (RCT) dan seterusnya mengurangkan risiko arteriosklerosis di dalam badan. Hasil yang memberangsangkan ini menunjukkan bahawa *Xestospongia* sp. mempunyai potensi untuk digunakan sebagai agen anti-arteriosklerotik.