

**IDENTIFICATION OF ANTI-ATHEROSCLEROTIC
COMPOUNDS FROM MARINE MOLLUSCS OF
BIDONG ARCHIPELAGO**

NUR MAISARAH BINTI SARIZAN

**MASTER OF SCIENCE
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**Thesis Submitted in Fulfillment of the
Requirement for the Degree of Master of
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To Mama & Abah

Your never ending love and support is a strength to me

Thank you for believing in me

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 : Faculty of Science and Technology

Atherosclerosis, which is a condition of thickening of the artery walls by the deposition of fatty material has been identified as one of the factors that cause the cardiovascular disease (CVD). Treatment for this disease includes using conventional drugs such as statins, niacins and fibrates, however, drugs targeting on high density lipoprotein (HDL) to reduce or prevent atherosclerosis are yet to be developed. This present study was focusing on finding new potential drugs from marine natural products using new targets for atherosclerosis treatment: Peroxisome Proliferator Response Element (PPRE) and Scavenger Receptor Class B Type 1 (SR-B1) promoters. Ten species of marine molluscs from Bidong archipelago have been used to study their bioactive compounds as potential anti-atherosclerotic substances: *Aplysia dactylomella*, *Chicoreus* sp., *Cypraea tigris*, *Hytissa* sp., *Lambis* sp., *Pedum* sp., *Pteria penguin*, *Tectus niloticus*, *Terebra muculata* and *Tridachna* sp.. The potential of these samples against atherosclerosis were investigated by using luciferase assays of PPRE and SR-B1 in HepG2 cells. Prior to that, cytotoxicity effects of samples were studied on HepG2 cells, and non-toxic samples were

selected for luciferase assay. One-way ANOVA was used to determine the luciferase activity of HepG2 cells after treatment with the samples, where results above the negative control value (1-fold) were considered as having potential. Results showed that all methanolic crude extracts (MCEs) screened for anti-atherosclerotic activities gave positive results at various effective concentrations (0.39 – 100.00 µg/mL). Two species with highest luciferase expression, *Chicoreus* sp. (4.96-fold) and *Tridachna* sp. (5.48-fold), were fractionized to give two different fractions, the diethyl ether fraction (DEF) and butanol fraction (BUF). Through the luciferase assays, both fractions were also showing positive results towards anti-atherosclerotic activities at their effective concentration with 1.56 µg/mL (1.84-fold) for DEF of *Tridachna* sp., 25.00 µg/mL (1.34-fold) for BUF of *Tridachna* sp., 12.50 µg/mL (1.51-fold) for DEF of *Chicoreus* sp., and 25.00 µg/mL (1.23-fold) for BUF of *Chicoreus* sp.. One active compound named as compound C35 was successfully isolated from the BUF of *Chicoreus* sp.. Through the GC-MS spectroscopy, C35 might be hexadecanoic acid methyl ester. This compound showed a non-toxic result towards the HepG2 cell lines (>100 µg/mL) and positive results for anti-atherosclerotic activities towards both promoters PPRE and SR-B1 with highest promoter expression at the same effective concentration, 12.50 µg/mL of 3.54- and 2.08-fold, respectively. Overall, this study shows that generally marine molluscs have potential as anti-atherosclerotic substances and more new active drugs from these molluscs should be discovered.

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**PENGENALANPASTIAN SEBATIAN-SEBATIAN ANTI-ATEROSKLEROTIK
DARI MOLUSKA MARIN DARI KEPULAUAN BIDONG**

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Jun 2013

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Aterosklerosis, iaitu keadaan di mana dinding salur darah menebal disebabkan oleh pemendapan bahan-bahan berlemak telah dikenalpasti sebagai salah satu faktor yang menyebabkan penyakit kardiovaskular (CVD). Rawatan untuk penyakit ini termasuklah menggunakan ubatan konvensional seperti statin, niasin dan fibrat, walau bagaimanapun, ubat yang mensasarkan 'high density lipoprotein' (HDL) untuk merendah atau mencegah aterosklerosis masih belum dicipta. Kajian ini tertumpu kepada mencari ubatan baru yang berpotensi dari produk semulajadi marin menggunakan sasaran baru dalam rawatan aterosklerosis: promoter 'Peroxisome Proliferator Response Element' (PPRE) dan 'Scavenger Receptor Class B Type 1' (SR-B1). Dalam kajian ini, sepuluh spesis moluska marin dari Kepulauan Bidong telah digunakan untuk mengkaji sebatian-sebatian bioaktif yang berperanan sebagai bahan yang berpotensi sebagai anti-aterosklerotik: *Aplysia dactylomella*, *Chicoreus* sp., *Cypraea tigris*, *Hyotissa* sp., *Lambis* sp., *Pedum* sp., *Pteria penguin*, *Tectus niloticus*, *Terebra muculata* dan *Tridachna* sp.. Potensi sampel-sampel ini bertindak terhadap aterosklerosis telah dikaji dengan menggunakan asai lusiferase menggunakan

promoter PPRE dan SR-B1 dalam sel HepG2. Sebelum itu kajian sitotoksik sampel ke atas sel HepG2 telah dijalankan dan sampel yang tidak toksik dipilih untuk asai lusiferase. ANOVA-sehala telah digunakan untuk menentukan aktiviti lusiferase dalam sel HepG2 selepas dirawat dengan sampel-sampel tersebut, di mana keputusan yang melebihi nilai kawalan negatif (1-gandaan) telah dipertimbangkan sebagai mempunyai potensi. Keputusan menunjukkan semua ekstrak kasar metanol (MCEs) yang telah disaring untuk aktiviti anti-aterosklerotik memberikan keputusan positif pada pelbagai kepekatan efektif (0.39 – 100.00 µg/mL). Dua spesis dengan ekspresi lusiferase tertinggi, *Chicoreus* sp. (4.96-gandaan) dan *Tridachna* sp. (5.48-gandaan), telah difraksi kepada dua fraksi berbeza: fraksi dietil eter (DEF) dan fraksi butanol (BUF). Melalui asai-asai lusiferase, kedua-dua fraksi juga telah menunjukkan keputusan aktiviti anti-aterosklerotik yang positif pada kepekatan efektif dengan 1.56 µg/mL (1.84-gandaan) bagi DEF dari *Tridachna* sp., 25.00 µg/mL (1.34-gandaan) bagi BUF dari *Tridachna* sp., 12.50 µg/mL (1.51-gandaan) bagi DEF dari *Chicoreus* sp., dan 25.00 µg/mL (1.23-gandaan) bagi BUF dari *Chicoreus* sp.. Satu sebatian aktif yang dinamakan sebagai sebatian C35 telah diasingkan dengan jayanya dari BUF dari *Chicoreus* sp.. C35 berkemungkinan adalah asid heksadekanoik metil ester melalui spektra GC-MS. Sebatian ini telah menunjukkan keputusan tidak-toksik terhadap sel HepG2 (>100 µg/mL) dan keputusan positif kepada aktiviti anti-aterosklerotik terhadap kedua-dua promoter PPRE dan SR-B1 dengan ekspresi promoter tertinggi adalah pada kepekatan efektif yang sama, 12.50 µg/mL pada 3.54- dan 2.08-gandaan, masing-masing. Keseluruhannya, kajian ini menunjukkan yang secara umum moluska marin mempunyai potensi sebagai bahan anti-aterosklerotik dan lebih banyak ubatan baru dari moluska ini perlu diterokai.