NURUL FAZIANA KAMAL MASTER OF SCIENCE 2013

EXTRACTS OF SPONGE-DERIVED FUNGI AS POTENTIAL ANTI-ATHEROSCLEROTIC AGENT

NURUL FAZIANA BINTI KAMAL

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2013

EXTRACTS OF SPONGE-DERIVED FUNGI AS POTENTIAL ANTI-ATHEROSCLEROTIC AGENT

NURUL FAZIANA BINTI KAMAL

Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in the School of Fundamental Science Universiti Malaysia Terengganu

October 2013

***

For my mom's hopes and my dad's sweat.
For my sisters' and my brother's support.
For my kind supervisor, Assoc. Prof. Mariam Taib.
For Islam and for my beloved Malaysia.

***

***

With Him are the keys of the unseen, the treasures that none knoweth but He. He knoweth whatever there is on the earth and in the sea. Not a leaf doth fall but with His knowledge: there is not a grain in the darkness (or depths) of the earth, nor anything fresh or dry (green or withered), but is (inscribed) in a record clear (to those who can read).

[Koran, 6: 59]

**

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

Extracts of Sponge-derived Fungi as Potential Anti-atherosclerotic Agent

Nurul Faziana binti Kamal

October 2013

Main Supervisor : Assoc. Prof. Mariam binti Taib, Ph.D.

School : Fundamental Science

Current drugs in the market for atherosclerosis mostly involved the reduction of atherosclerotic progression by decreasing the plasma low density lipoprotein (LDL) levels. With prolonged use of this drug, it is reported to may cause liver failure. Meanwhile, drugs targeting on high density lipoprotein (HDL) in reducing/preventing the development of atherosclerosis are yet to be developed, although HDL plays an important role in reverse cholesterol transport (RCT). Potential targets in RCT have been identified to prevent atherosclerosis: scavenger receptor class B type 1 (SR-B1) and peroxisome proliferator response elements (PPRE). This study was therefore carried-out to explore new peroxisome proliferator activated receptor (PPAR) ligand agonists for the activation of these potential targets, from sponge-derived fungi, as potential drugs for atherosclerosis. Sponges were collected from Bidong archipelago and fungi associated with the sponges were then isolated. A total of 67 individuals were isolated from *Xestospongia* sp., *Aaptos* sp., and *Theonella swinhoei* and identification resulted in only six species: *Aspergillus flavus*, *Aspergillus terreus, Aspergillus niger*, *Penicillium* sp., *Trichoderma hamatum* and *Eupenicillium japanicum.* Ethyl acetate (EA) extracts of all six isolates were prepared and cytotoxicity test of extracts on human hepatocellular carcinoma cells (HepG2) were carried-out; non-cytotoxic extracts were chosen since viable HepG2 cells were needed later to transfect with Luciferase reporter plasmids. Only extract of *A. flavus* showed cytotoxic effect towards HepG2 cells (IC_{50}=3.88±2.31 µg/ml) while other extracts displayed non-cytotoxic effect: *A. terreus* (IC_{50}= 94.19±12.13 µg/ml) and *A. niger* (40.27±7.82 µg/ml); IC_{50} values for *Penicillium* sp., *T. hamatum* and *E. japanicum* extracts cannot be detected. For anti-atherosclerotic

activity, the effect of fungal extracts on regulating the transcriptional activity of PPRE and SR-B1 were determined. Increase the transcriptional activity of Luciferase reporter reflects the increase of the expression of PPRE or SR-B1. HepG2 cells transfected with PPRE-Luciferase reporter plasmid and SRB1-Luciferase reporter plasmid were treated with fungal extracts of *A. terreus, Penicillium* sp., *T. hamatum,* and *E. japanicum.* Results of PPRE-Luciferase assay showed that *T. hamatum* induced highest transcriptional activity of the PPRE as well as activity of SR-B1 promoter in SRB1-Luciferase assay. Preliminary detection of secondary metabolite in *T. hamatum* with potential anti-atherosclerotic activity was carried-out using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). TLC results showed that *T. hamatum* extract consists no alkaloids but it might consist compound with C=C double bond, sugar and phenylhydrazones-based compounds. While HPLC result had detected five peaks which suggesting five different compounds in this extract. However, the exact components in this extract cannot be determined yet. Extract of *T. hamatum* can become potential anti-atherosclerotic agent with PPRE and SR-B1 as targets. This fundamental study can provide useful information for other researchers to discover the maximum potential of marine fungi as anti-atherosclerotic agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Master Sains.

Potensi Ekstrak Kulat Terbitan Span Sebagai Agen Anti-arterosklerotik

Nurul Faziana binti Kamal

October 2013

Penyelia Utama : Prof. Madya Mariam binti Taib, Ph.D.

Pusat Pengajian : Sains Asas

Ubat-ubatan terkini di pasaran bagi merawat arterosklerosis kebanyakannya melibatkan penurunan kadar plasma lipoprotein berketumpatan rendah (LDL) di mana penggunaan ubat ini secara berterusan boleh menyebabkan kegagalan fungsi hati. Walaupun lipoprotein berketumpatan tinggi (HDL) memainkan peranan penting dalam pengangkutan kolesterol berbalik (RCT), namun ubat-ubatan yang menjadikan HDL sebagai sasaran dalam mencegah penyakit arterosklerosis masih lagi dalam peringkat pembangunan. Sasaran utama dalam RCT yang dikenalpasti bagi pencegahan arterosklerosis ialah reseptor penggerut kelas B jenis 1 (SR-B1) dan elemen rangsangan peroksisom peroliferasi (PPRE). Oleh itu, kajian ini dilakukan bagi mencari agonis baru kepada ligan *peroxisome proliferator activated receptor* (PPAR) daripada kulat span, untuk mengaktifkan sasaran tersebut sebagai rawatan berpotensi dalam pencegahan arteriosklerosis. Span dari gugusan Pulau Bidong telah diperoleh dan kulat yang bersekutu dengannya telah berjaya dipencilkan. Sejumlah 67 individu kulat telah dipencilkan daripada jenis spesis *Xestospongia* sp., *Aaptos* sp., dan *Theonella swinhoei.* Daripada ini, hanya enam spesis kulat telah dikenalpasti: *Aspergillus flavus*, *Aspergillus terreus, Aspergillus niger*, *Penicillium* sp., *Trichoderma hamatum* dan *Eupenicillium japanicum.* Ekstrak etil asetat untuk kesemua jenis kulat telah disediakan dan ujian ketoksikan ekstrak terhadap sel hati manusia (HepG2) telah dijalankan; ekstrak yang tidak toksik telah dipilih memandangkan sel HepG2 yang hidup diperlukan untuk ditransfeksi dengan plasmid pelapor Luciferase. Hanya ekstrak *A. flavus* menunjukkan kesan toksik terhadap sel HepG2 (IC_{50}=3.88±2.31 µg/ml) manakala ekstrak yang lain menunjukkan kesan tidak toksik terhadap sel: *A. terreus* (IC_{50}= 94.19±12.13 µg/ml) dan

A. niger (40.27±7.82 µg/ml); manakala nilai IC_{50} untuk ekstrak *Penicillium* sp., *T. hamatum* dan *E. japanicum* tidak dapat ditentukan. Bagi aktiviti anti- arterosklerosis, kesan ekstrak fungi terhadap aktiviti transkripsi PPRE dan SR-B1 telah ditentukan. Peningkatan aktiviti transkripsi pelapor Luciferase juga memberi maksud peningkatan ekspresi PPRE atau SR-B1. Sel HepG2 yang telah ditransfeksi dengan plasmid pelapor Luciferase-PPRE dan plasmid pelapor Luciferase-SRB1 telah dirawat dengan ekstrak kulat *A. terreus, Penicillium* sp., *T. hamatum,* dan *E. japanicum.* Keputusan asai PPRE-Luciferase menunjukkan bahawa *T. hamatum* telah menghasilkan aruhan yang tinggi ke atas aktiviti transkripsi PPRE dan juga aktiviti trankripsi promoter SR-B1 dalam asai SRB1-Luciferase. Pengesanan awal metabolit sekunder dalam ekstrak *T. hamatum* yang mempunyai potensi sebagai anti- arterosklerosis telah dijalankan dengan menggunakan kromatografi lapisan nipis (TLC) dan kromatografi cecair berprestasi tinggi (HPLC). Keputusan TLC menunjukkan ekstrak *T. hamatum* tidak mengandungi alkaloid tetapi mungkin mengandungi komponen dengan ikatan ganda dua C=C, gula dan unsur berasaskan fenilhidrazan. Manakala, keputusan HPLC telah mengesan lima jenis komponen yang berbeza. Walaubagaimanapun, komposisi komponen dalam ekstrak ini masih tidak dapat ditentukan lagi. Ekstrak *T. hamatum* berpotensi sebagai agen anti-arterosklerotik dengan menjadikan PPRE dan SR-B1 sebagai sasaran. Kajian ini diharap dapat memberi maklumat berguna kepada penyelidik lain untuk meneroka potensi kulat marin secara maksimum sebagai agen anti-ateriosklerotik.