

IDENTIFICATION OF BIOACTIVE COMPOUNDS  
FROM *Acanthaster planci* AND THEIR ANTI-  
ATHEROSCLEROTIC ACTIVITY

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and their anti-atherosclerotic activity / Nurul Hazirah Mat Lazim  
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IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM  
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ATHEROSCLEROTIC ACTIVITY

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Thesis submitted in fulfillment of the Requirements for the  
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Technology  
Universiti Malaysia Terengganu

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## DEDICATION

Dedicated with love...

To my parents, Mat Lazim & Mastura....

To my sisters and brother, Angah, Efi & Adik...

And to my future groom to be...Mohd Adib Farhan..

## ABSTRACT

### Identification of Bioactive Compounds from *Acanthaster planci* and Their Anti-atherosclerotic Activities

Nurul Hazirah Mat Lazim @ Mahasan

June 2013

**Chairperson :** Asnuzilawati Asari, Ph. D.

**Member :** Faridah Mohamad, Ph.D.  
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**Faculty :** Science and Technology

Echinoderms have been reported to show biological activities like antifouling and antibacterial properties. Therefore this study was conducted to isolate and characterise the secondary metabolites from *Acanthaster planci* and evaluate them for toxicity and atherosclerosis assay. The isolation of extract and fractions were carried out with using outer layer of *Acanthaster planci* (APOL) using vacuum liquid chromatography, column chromatography, chromatotron and high performance liquid chromatography (HPLC) yielding six compounds. Methyl benzoate (KBKR-22) was isolated from butanol fraction and (1-[4-(6-propyl-3,4,5,6-tetrahydro-pyridazin-3-yl)-phenyl]-ethanone) (KK1-R1-5) was isolated from methanolic extract. Three sterol skeletal compounds, KBKR-2, KK1-R1-15 and EK-8 were isolated from butanol fraction, methanolic extract and diethyl ether fraction respectively. P5 was isolated from diethyl ether fraction using HPLC. However, the characterisation could not be done because of insufficient of compound.

For biological properties of sample from echinoderms, the antibacterial properties was determined using disc diffusion test (DDT) and toxicity using MTS (tetrazolium salt). Methanolic extracts of echinoderms inhibited two strains of gram positive bacteria tested i.e. *Streptococcus uberis* and *Klebsiella pneumoniae*, showing species preference.

All extracts and fractions were not toxic towards HepG2 cells with the IC<sub>50</sub> value were higher than 30µg/mL indicating good potential as anti-atherosclerotic agent. These extracts and fractions were subjected to Luciferase assay (PPRE) to determine their potential as ligand for the prevention of atherosclerosis. They showed good luciferase activity by expressing the luciferase gene.

The isolated compounds were subjected to Luciferase assay with PPRE and SRB-1 promoter in order to confirm their ability to act as ligand to prevent atherosclerosis. From six isolated compounds, KBKR-22 expressed the highest luciferase activity (SRB-1; more than 2 fold compared to positive control) at concentration of 3.12µg/ml.

Thus, echinoderms showed good potential as anti-atherosclerotic agent and *Acanthaster planci* is an excellent candidate for prevention atherosclerosis study by offering many potential compounds for that purpose.

# ABSTRAK

## Pencirian Sebatian Bioaktif daripada *Acanthaster planci* dan Aktiviti Anti-aterosklerotik

Nurul Hazirah Mat Lazim @ Mahasan

Jun 2013

**Pengerusi** : Asnuzilawati Asari, Ph. D.

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Ekstrak daripada Echinoderms telah menunjukkan aktiviti biological seperti kebolehan sebagai agen anti pelekatan organisma dan anti bakteria. Maka, kajian ini dilakukan untuk memencilkan dan mencirikan metabolit sekunder daripada *Acanthaster planci* dan dianalisa untuk ketoksikan dan assai aterosklerosis. Pemencilan sebatian telah dilakukan kepada ekstrak dan fraksi daripada lapisan luar *Acanthaster planci* dengan menggunakan kromatografi cairan vakum, kromatografi kolum, kromatotron dan HPLC dan menghasilkan enam sebatian. Metil benzoate (KBKR-22) telah dipencilkan daripada fraksi butanol dan (1-[4-(6-propyl-3,4,5,6-tetrahydro-pyridazin-3-yl)-phenyl]-ethanone) (KK1-R1-5) dipencilkan daripada ekstrak metanol. Tiga sebatian rangka sterol, KBKR-2, KK1-R1-15 dan EK-8 telah masing-masing dipencilkan daripada fraksi butanol, ekstrak methanol dan fraksi dietil eter. P5 dipencilkan daripada fraksi dietil eter menggunakan HPLC.

Walaupun bagaimanapun, pencirian tidak dapat dilakukan akibat kekurangan jumlah sampel.

Untuk aktiviti biologikal, sifat antibakteria ditentukan menggunakan ujian cakera resapan (DDT) dan ketoksikan menggunakan garam tetrazolium MTS. Ekstrak metanol merencat pertumbuhan dua jenis bakteria; *Streptococcus uberis* dan *Klebsiella pneumoniae*, merujuk kepada spesis.

Semua ekstrak dan fraksi adalah tidak toksik terhadap sel HepG2 menunjukkan potensi yang bagus sebagai agen anti-aterosklerotik. Ekstrak dan fraksi ini telah diuji terhadap assai Luciferase (PPRE) bagi menentukan potensi sebagai ligan untuk mencegah aterosklerosis. Semua ekstrak menunjukkan aktiviti Luciferase yang baik.

Sebatian-sebatian yang dipencilkan telah diuji untuk assai PPRE and SRB-1 bagi mengesahkan kebolehan untuk bertindak sebagai ligan bagi mencegah aterosklerosis. Daripada enam sebatian, KBKR-22 mengekpres aktiviti Luciferase yang paling tinggi (SRB-1; lebih daripada 2 gandaan berbanding kawalan positif) pada kepekatan of 3.12 $\mu$ g/ml.

Maka, ekinoderma telah menunjukkan potensi yang bagus sebagai ejen anti-aterosklerotik dan *Acanthaster planci* merupakan calon terbaik untuk kajian pencegahan aterosklerosis dengan menawarkan pelbagai sebatian yang berpotensi untuk tujuan tersebut.