

CHARACTERIZATION OF ANTIFOULING
METABOLITES FROM BOOK GILLS AND
CARAPACE OF HORSESHOE CRAB,
Tachypleus gigas

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MASTER OF SCIENCE
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MUHAMAD SYUKERI BIN AHMAD

**Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of
Science in Institute of Marine Biotechnology
Universiti Malaysia Terengganu
2017**

Dedicated this thesis to:

My supervisor, Associate Professor Noraznawati Ismail, PhD.

My beloved parents and siblings

My friends

and lastly, P.N.H

For all their dedication, sacrifice and endless love

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

CHARACTERIZATION OF ANTIFOULING METABOLITES FROM BOOK GILLS AND CARAPACE OF HORSESHOE CRAB, *Tachypleus gigas*

MUHAMAD SYUKERI BIN AHMAD

November 2016

Main Supervisor : Associate Professor Noraznawati Ismail, PhD

Institute : Institute of Marine Biotechnology

Horseshoe crabs have roamed the world for almost 450 million years since the first fossil was discovered. Their survivality were due to various adaptations and potent defence system; in which hemolymph is one of them. Few studies emphasize on the horseshoe crabs book gills and carapace. The carapace was known as the armour that protects the soft tissue below them. Despite being submerged almost all the time and has a flat surface, the horseshoe crabs seem not to be affected much by biofouling phenomena. The objectives of this study were to determine the metabolite profiles of the book gills and carapace extracts, to identify the antifouling activities they possess and to characterize the metabolites that contributes to the antifouling activities. In this study, book gills and carapace crude extracts were screened for antifouling activities along with their modes of actions; detachment and preventive actions. Fractionations were conducted after the modes of action of the antifouling activities has been determined.

The components of the extracts that exhibits significant activities were analysed using thin layer chromatography, recycling high performance liquid chromatography and gas chromatography-mass spectrometry to obtain their chemical profiles. It was found that book gills and carapace extracts possess significant antifouling activities and exhibits chemical interactions such as synergism and antagonism effects that plays a major role in their activity levels. The book gills and carapace extracts exhibit significant detachment actions ($\geq 40\%$). Both extracts possess similar chemical profiles which composed of terpenoids, phenols and organic acids such as simple sugar and amino acids. These organic acids were found to be the key to the detachments actions due to the nutrient-induced biofilm dispersal mechanism. In conclusion, both book gills and carapace possess antifouling activities, which might explain their ability to remove attached fouling organisms. Further analysis should be done for deeper understanding regarding to their defence mechanisms. This may leads to discovery of new biofilm dispersal agents in near future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN METABOLIT ANTI BIO-LEKATAN DARIPADA INSANG BUKU DAN KARAPAS BELANGKAS, *Tachypleus gigas*

MUHAMAD SYUKERI BIN AHMAD

November 2016

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Belangkas telah hidup hampir 450 juta tahun sejak fosil pertama ditemui. Kemandirian spesies mereka adalah disebabkan kebolehan mereka untuk beradaptasi dan sistem pertahanan badan mereka yang kuat, antaranya ialah hemolimfa yang telah dikaji dari pelbagai aspek. Anggota- anggota badan lain pada belangkas kurang diberikan perhatian dalam kajian tentang sistem pertahanan semula jadi mereka, antaranya ialah insang buku dan karapas. Insang buku ialah organ pernafasan manakala karapas ini dikenali sebagai perisai yang melindungi tisu-tisu lembut di bawah mereka. Walaupun insang buku dan karapas sentiasa berada di dalam air dan berisiko tinggi terhadap fenomena biofilem, mereka seolah-olah tidak terjejas dengan masalah ini. Antara objektif- objektif kajian ini ialah menentukan profil metabolit ekstrak buku insang dan karapas, untuk mengenal pasti aktiviti-aktiviti anti bio-lekatan yang mereka miliki dan untuk mencirikan metabolit yang menyumbang kepada aktiviti anti bio-lekatan.

Ekstrak insang buku dan karapas telah disaring untuk aktiviti anti bio-lekatan menggunakan kaedah bioasai kristal ungu. Komponen ekstrak yang mempamerkan aktiviti yang signifikan seterusnya dianalisis menggunakan kromatografi lapisan nipis, kitar semula kromatografi cecair berprestasi tinggi dan gas kromatografi-spektrometri jisim untuk mendapatkan profil kimia mereka dan mencirikan komponen yang aktif. Didapati bahawa kedua-dua ekstrak mempunyai aktiviti anti bio-lekatan yang ketara dan menunjukkan interaksi kimia seperti kesan sinergi dan kesan kepentingan bertentangan yang memainkan peranan utama dalam menentukan tahap aktiviti mereka. Ekstrak mempamerkan mod tindakan penyahlekatan. Kedua-dua ekstrak mempunyai profil kimia yang sama terdiri daripada terpenoid, fenol dan asid organik seperti gula dan asid amino. Asid organik didapati kunci kepada tindakan anti bio-lekatan, mungkin disebabkan oleh mekanisme penyahlekatan biofilem yang disebabkan oleh kandungan nutrisi yang berlebihan yang mendorong bakteria untuk berpisah daripada biofilemnya. Kesimpulannya, kedua-dua insang buku dan karapas mempunyai aktiviti anti bio-lekatan yang mungkin menjelaskan kemampuan mereka untuk mengatasi masalah biofilem. Analisis lanjut untuk mencari sebatian penting lain yang perlu dilakukan untuk mengkaji mengenai mekanisme pertahanan badan mereka.