CHARACTERIZATION OF CRUDE EXTRACTS FROM HORSESHOE CRABS *TACHYPLEUS GIGAS* AND *CARCINOSCORPIUS ROTUNDICAUDA* IN MALAYSIA

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Despite the extensive study on this living fossil, most studies on horseshoe crab are focused on its blood which contains haemolymph and ameobocyte lysate. Less focus was given on the other part of the horseshoe crab which also has the equal potential in providing beneficial substances for the benefit of human. This study was dedicated to explore and determine other potential bioactivity derived from other than its blood. The carapace and the book gill of *Tachypleus gigas* and *Carcinoscorpius rotundicauda* were used in this research and selected solvents were used to obtained crude extract. The crude extracts were tested for the presence of antibacterial and antifouling activities. Selected crude extract with bioactivity was subjected for purification and identification using ^{1}H- NMR. Overall picture revealed that the book gills produced higher percentage of crude extract compared with carapace. Methanolic or solvents with higher polarity extracted more crude than other solvents. Both antibacterial and antifouling activities were present from the crude extracts. Most antibacterial activities were detected on Gram-

positive bacteria. The most vulnerable test strain towards the crude extract was *Staphylococcus aureus*. Acetone crude extract showed the highest number of inhibition on test strains. The highest zone of inhibition was displayed by acetone carapace crude extract on *S. typhimurium* which also displayed wide range of activity. Antifouling activity was present from the test conducted. *T. gigas* crude carapace and book gill showed more antifouling activity compared with *C. rotundicauda*. No marked difference between Gram-positive and negative observed towards crude extract from both carapace and book gills. However, carapace crude extract showed higher bioactivity compared with book gills while female book gill crude extract showed more bioactivity compared with male.

The compound that showed antifouling activity was polar in nature because aqueous and methanol extracts for both carapace and book gills accumulate higher bioactivity properties. The crude extract prepared in acetone with the carapace female of *C. rotundicauda* was destined for column chromatography and purified sterol were obtained after being identified through proton NMR. In conclusion, antibacterial and antifouling activities in carapace and book gills extracts were present and this could be added to the complexity of the defense mechanisms in horseshoe crab which supported innate immunity in the animal. Further investigation should be done to gain conclusive insight.

Abstrak tesis ini dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi syarat untuk Ijazah Sarjana Sains.

PENCIRIAN EKSTRAK KASAR DARI BELANGKAS *TACHYPLEUS GIGAS* DAN *CARCINOSCORPIUS ROTUNDICAUDA* DI MALAYSIA

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Pelbagai kajian telah dijalankan ke atas fosil hidup ini tetapi kebanyakkannya tertumpu pada darahnya yang mengandungi hemolimfa dan lisat amebosit. Perhatian kurang diberikan kepada bahagian badan belangkas lain yang mungkin mempunyai potensi yang sama penting dalam memberikan sebatian yang berguna untuk manusia. Kajian ini tertumpu kepada penerokaan dan penentuan potensi sebatian yang mempunyai bioaktiviti daripada sumber alternatif selain darahnya. Karapas dan buku insang dari spesis *Tachypleus gigas* dan *Carcinoscorpius rotundicauda* telah digunakan dalam kajian ini dan pelarut terpilih telah digunakan untuk mengekstrak sebatian mentah dari sampel tersebut. Ekstrak mentah telah diuji untuk menentukan kehadiran agen antibakteria dan juga ‘antifouling’. Ekstrak mentah terpilih yang menunjukkan akitiviti telah melalui proses penulenan dan dikenalpasti dengan menggunakan ^{1}H-NMR. Secara keseluruhannya, buku insang dari kedua-dua spesies menunjukkan peratusan ekstrak mentah yang lebih tinggi berbanding yang lain. Methanol dan pelarut yang

mempunyai polariti yang lebih tinggi mengekstrak sebatian mentah lebih tinggi berbanding pelarut lain. Aktiviti antibakteria dan antifouling telah dapat dikesan di dalam ekstrak mentah yang diperoleh. Kebanyakkan aktiviti antibakteria dikesan pada bakteria Gram-positif. Bakteria *S. aureus* ternyata adalah bakteria yang paling alah kepada ekstrak mentah belangkas. Ekstrak mentah pelarut aceton menunjukkan bilangan perencatan pertumbuhan tertinggi ke atas bakteria yang diuji. Ekstrak mentah pelarut aceton menunjukkan diameter perencatan tertinggi ke atas bakteria *S. typhimurium* yang juga menunjukkan jurang aktiviti yang luas. Terdapat juga aktiviti 'antifoling' dari kajian yang telah dilakukan. Ekstrak mentah aktiviti 'antifouling' karapas dan buku insang *T. gigas* menunjukkan lebih banyak aktiviti antifouling berbanding dengan *C. rotundicauda.* Tiada perbezaan yang ketara membezakan tahap kelemahan bakteria positif dan negatif terhadap ekstrak mentah kedua-dua karapas dan buku insang. Walaubagaimanapun, karapas menunjukkan aktiviti yang lebih tinggi berbanding buku insang manakala ekstrak mentah buku insang betina menunjukkan lebih aktiviti berbanding jantan.

Sebatian yang mempunyai aktiviti 'antifouling' mempunyai ciri-ciri polar kerana ekstrak akues dan methanol bagi karapas dan buku insang menunjukkan kehadiran bioaktiviti melebihi ekstrak lain. Sebatian ekstrak aceton karapas *C. rotundicauda* betina telah melalui proses kromatografi turus dan sterol tulen telah diperolehi setelah dikenalpasti menggunakan proton NMR. Sebagai kesimpulan, aktiviti antibakteria dan antifouling telah dapat dikenalpasti kehadirannya di dalam sebatian ekstrak dan ini dapat menambah maklumat terhadap kompleksiti mekanisma pertahanan di dalam

belangkas yang turut memiliki imuniti dalaman. Kajian selanjutnya wajar dilakukan untuk mengesahkan hasil kajian.