ISOLATION OF BIOACTIVE METABOLITES FROM MARINE SPONGES, *AAPTOS AAPTOS* AND *CELTODORYX GIRARDAE*

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To my beloved Mother, Che Anah Derasa and
Father, Mat Rashid Ab. Rahman

&

My siblings

For your infinite and unfading love, sacrifice, patience,
encouragement and

Best wishes

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

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In this study, samples from *Aaptos aaptos* collected from the coastal waters of Terengganu, Malaysia, were investigated for antibacterial (disc diffusion method), antioxidant (DPPH free radical scavenging method), antiviral (neutral red uptake method) and cytotoxicity (MTT method) as well as the study of mechanism of cancer cell death. On the other hand, polysaccharide from *Celtodoryx girardae* discovered in the Golfe du Morbihan, France and its symbiotic bacteria underwent biochemical analysis, antiviral (neutral red uptake method) as well as mechanism of antiviral action investigation.

In antibacterial activity of 12 *A. aaptos* crude extracts (CEs), moderate bacterial inhibitions were showed by 25% samples against *B. subtilis*, 8% against *B. proteus*, 33% against *Streptococus* sp., 33% against *S. agalatea* and 25% showed weak activity against *E. coli*. Meanwhile, CEs showed strong radical scavenging activity, despite lower activity (<90%) in comparison to standards.

Besides that, the chloroform (ABCE), ethyl acetate (ABEE) and methanol (ABME) solvent fractions showed similarity in higher proportions of straight chain saturated fatty acids (FAs), made up of about 28.5 (16:0), 46.4 (22:0) and 40.9 (23:0) % of the total FA content, respectively, while hexane extract (ABHE) was dominated by unsaturated FAs which made up of 36%. Several CEs also showed cytotoxicity towards HL-60 and MCF-7 cell lines. ABCE were particularly active towards the cell lines HL-60, MCF-7, K562, CEM-SS and WEHI-3B with CD_{50} values ranging from 3.0 to 21.0 μg/mL. ABCE were also capable to inhibit the *in vitro* replication of *Herpes simplex* Virus type 1 (HSV-1) in Vero cells with 50% inhibitory concentration (EC_{50}) value of 60.5 μg/mL while exhibiting weak cytoxicity effect against normal Vero cells (≤10% of cell destruction).

The bioassay-guided isolation have resulted the purification of known compounds, a sterol (5α-cholestan-3β-ol; A1) and a sterol ester (cholestanyl myristate; A2) from hexane extract, as well as an alkaloid (aaptamine; A4) and a new derivative of the alkaloid (A5), from the bioactive chloroform extract. Detailed analysis by NMR and mass spectroscopy enabled A5 identification to be 3-(phenethylamino)demethyl(oxy)aaptamine. Only cytotoxic activities of the two alkaloids (A4 and A5) were further evaluated against HL-60 and WEHI-3B due to the insolubility of A1 and A2 in DMSO. A4 and A5 exhibited strong cytotoxic activity against HL-60 (CD_{50} values; 1.1 ± 0.1 μg/mL and 5.2 ± 1.6 μg/mL respectively) while each showed strong and moderate activity against WEHI-3B (CD_{50} values; 3.4 ± 0.4 μg/mL and 19.4 ± 3.8 μg/mL respectively).

The morphological assessment through observation of light microscopy and fluorescence microscopy (AO/PI double staining analysis), revealed that mode of cell death (HL-60 and WEHI-3B) induced by A4 and A5 was through apoptosis mechanism. In investigation of anti-HSV-1 activity and cytotoxicity against normal Vero cell, only chloroform extract and A4 showed moderate and strong anti-HSV-1 activity (EC_{50} values; 60.5 and 7.0 μg/mL respectively) while showing weak cytoxicity effect (≤10% of cell destruction).

On the other hand, exopolysaccharides (EPS) were extracted from marine sponge, *Celtodoryx girardae*. The sponge samples were collected monthly from November 2007 to May 2008. Size exclusion chromatography (SEC) isolation and analysis of EPS samples from *C. girardae* and its symbiotic bacteria yielded a similar unique molecular weight of ≈ 800 kDa. However, the infrared analysis revealed that EPS structural variations occurred in term of their sources and seasonal dependent.

EPS fractions have exhibited significant sulfate contents and were screened *in vitro* for potential antiviral activity against HSV-1. The best result was obtained from a sample collected in January, SJan-3 which exhibits an EC_{50} of 5.9 μg/mL without cytotoxicity effect on the Vero cell line. The experiments which carried out to elucidate the mechanism of EPS (SJan-3) antiviral action showed that the sulfated groups of EPS interact with the glycoproteins on the surface of the virus membrane.

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

PEMENCILAN BIOAKTIF METABOLIT DARIPADA SPAN LAUT, *AAPTOS AAPTOS* DAN *CELTODORYX GIRARDAE*

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Dalam kajian ini, sampel daripada *Aaptos aaptos* yang dikumpul daripada perairan Terengganu, Malaysia, telah dikaji untuk antibakteria (kaedah pembauran cakera), antioksida (pemerangkapan radikal bebas oleh DPPH), antiviral (kaedah pengambilan dakwat neutral merah) dan aktiviti ketoksikan (kaedah MTT) serta kajian mekanisma kematian sel kanser. Sementara itu, polisakarida daripada *Celtodoryx girardae* yang dikumpul daripada Teluk Morbihan, Perancis, serta daripada bakteria yang bersimbiosis dengannya pula melalui analisis biokimia, ujian antiviral (kaedah pengambilan dakwat neutral merah) dan pengenalpastian mekanisma tindakan antiviral.

Dalam ujian untuk potensi 12 ekstrak mentah sebagai antibakteria, perencatan yang sederhana ditunjukkan oleh 25% sampel terhadap *B. subtilis*, 8% terhadap *B. proteus*, 33% terhadap *Streptococus* sp., 33% terhadap *S. agalatea* dan 25% sampel menunjukkan perencatan yang lemah terhadap *E. coli*. Sementara itu,

semua ekstrak menunjukkan aktiviti pemerangkapan radikal bebas yang tinggi walaupun lebih rendah (<90% pemerangkapan) berbanding kawalan yang digunakan. Selain itu, ekstrak kloroform, etil asetat dan methanol menunjukkan persamaan dalam kandungan asid lemak rantai lurus meliputi 28.5 (16:0), 46.4 (22:0) and 40.9 (23:0) % daripada jumlah keseluruhan kandungan asid lemak, sementara ekstrak heksana lebih didominasi oleh asid lemak tak tepu (ALTT) dengan amaun 36%.

Beberapa ekstrak mentah juga menunjukkan potensi ketoksikan terhadap HL-60 dan MCF-7. Ekstrak kloroform menunjukkan aktiviti ketoksikan yang meyakinkan terhadap sel-sel kanser HL-60, MCF-7, K562, CEM-SS dan WEHI-3B dengan nilai CD_{50} dari julat 3.0 ke 21.0 μg/mL. Ekstrak kloroform juga berpotensi merencatkan replikasi secara *in vitro* Virus *Herpes simplex* jenis 1 (HSV-1) dengan 50% nilai perencatan ialah 60.5 μg/mL disamping menunjukkan kesan ketoksikan lemah terhadap sel Vero normal (≤10% kemusnahan sel).

Pemencilan kandungan bioaktif berpandukan keputusan bioasai telah berjaya memencilkanan komponen yang telah dikenali, sterol (5α-cholestan-3β-ol; A1) dan sterol ester (cholestanyl myristate; A2) daripada ekstrak heksana, begitu juga alkaloid (aaptamine; A4) dan satu terbitan alkaloid baru (A5) telah dipencilkan daripada ekstrak bioaktif kloroform. Analisis terperinci melalui NMR dan MS membolehkan pengenalpastian A5 sebagai 3-(phenethylamino)-demethyl(oxy)aaptamine. Hanya aktiviti ketoksikan oleh dua alkaloid (A4 dan

A5) terhadap HL-60 dan WEHI-3B yang terus dikaji memandangkan ketidaklarutan A1 dan A2 dalam DMSO. Hasilnya, A4 dan A5 menunjukkan aktiviti ketoksikan yang kuat terhadap HL-60 (nilai CD_{50} masing-masing; 1.1 ± 0.1 μg/mL dan 5.2 ± 1.6 μg/mL) sementara setiap satu menunjukkan aktiviti yang kuat dan sederhana terhadap WEHI-3B (nilai CD_{50} masing-masing; 3.4 ± 0.4 μg/mL dan 19.4 ± 3.8 μg/mL).

Penilaian secara morfologi melalui pemerhatian daripada mikroskop cahaya dan mikroskop floresen (analisis pewarnaan AO/PI) menunjukkan mekanisma kematian sel (HL-60 dan WEHI-3B) diaruhkan oleh A4 dan A5 adalah melalui apoptosis. Dalam kajian aktiviti anti-HSV-1 dan ketoksikan terhadap sel Vero normal, hanya ekstrak kloroform dan sebatian A4 masing-masing menunjukkan aktiviti anti-HSV-1 yang sederhana dan kuat (nilai EC_{50} masing-masing; 60.5 dan 7.0 μg/mL) di samping hanya memberi kesan ketoksikan yang lemah (≤10% kemusnahan sel).

Sementara itu, eksopolisakarida (EPS) telah diekstrak daripada span *Celtodoryx girardae*. Sampel span telah dikumpulkan secara bulanan daripada November ke May 2008. Pemencilan dan analisis SEC terhadap sampel-sampel EPS daripada daripada *Celtodoryx girardae* dan bakteria yang bersimbiosis dengannya telah memberikan hasil polisakarida tulen dengan berat molekul yang sama ≈ 800 kDa. Walau bagaimanapun, analisis infra-merah menunjukkan terdapat kepelbagaian struktur EPS mengikut sumber dan musim.

Analisis komponen kimia fraksi-fraksi EPS tulen tersebut telah menunjukkan terdapat kandungan sulfat yang signifikan dan fraksi-fraksi tersebut telah disaring secara *in vitro* untuk ujikaji antiviral terhadap *Herpes simplex* virus jenis 1 (HSV-1). Keputusan terbaik diperolehi daripada sampel yang dikumpul dalam bulan Januari, SJan-3 yang menunjukkan nilai EC_{50} 5.9 µg/mL tanpa memberi kesan ketoksikan pada sel Vero normal. Eksperimen yang telah dijalankan untuk menentukan mekanisma antiviral EPS (SJan-3) menunjukkan berlakunya tindakbalas antara kumpulan sulfat EPS dengan glikoprotein pada permukaan membran virus.