STRUCTURAL DIVERSITY AND BIOLOGICAL PROPERTIES OF SECONDARY METABOLITES FROM SEA HARE (*APLYSIA DACTYLOMELA*) AND ALCYONACEAN SOFT CORALS

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ABSTRACT

North Borneo, located in the Coral Triangle Region (CTR), the world's marine mega biodiversity, has an extremely rich source of marine life. The marine ecosystem is a fusion of complex interaction between the marine environment and marine organisms leading to predatory, competition for space or habitat, protection from disease or infection and directly imposing the pressure to survive among slow-moving, sessile soft bodied marine invertebrates. Thus, marine invertebrates biosynthesize secondary metabolites as a means of self defence. Recent development in drug discovery has revealed the potential of marine secondary metabolites as lead pharmaceutical drugs. This research focuses on two invertebrates; the sea hare *Aplysia dactylomela* Rang and *Alcyonacean* soft corals (*Lobophytum pauciflorum*, *Sinularia flexibilis* and *Scleronephthea corymbosa*) to investigate the structural diversity of secondary metabolites and its biological potentials. Populations of *A. dactylomela* from Dinawan Island yielded 10 compounds, Sulug Island yielded 9 compounds and Mantanani Island yielded 12 compounds, comprising of five new compounds with two new chemical skeletons. Compounds were mostly halogenated and comprised of acetogenins, charmigrane, cuparane, syndrean and bromoindoles. Three *Alcyonacean* soft coral species were analysed to contain 22 secondary metabolites; *Lobophytum pauciflorum* yielded 6 compounds, *Scleronephthya corymbosa* yielded 6 compounds and *Sinularia flexibilis* yielded 10 compounds comprising of furanocembranoids, pregnane type strerols and cembrane diterpenes. Secondary metabolites from the soft corals yielded five new compounds. Structure elucidation of compounds was determined via 1D-NMR (proton and carbon), 2D-NMR (HSQC, COSY, HMBC and NOESY), HRMS and FTIR measurements. Sesquiterpenes (+)-elatol (***16***) and 2,3,5,6-tetrabromoindole (***22***) inhibited the growth of *Escherichia coli* (HP0408) and *Vibrio cholera* (HP0908) at MIC 5 μg/mL and 10 μg/mL. The syndrean 12-acetoxypalisadin B (0.02 %)(***13***) and pregnane sterol P3N-4 (0.01 %) (***32***) displayed cytotoxicity towards HL60 (13 μg/mL) and B16F10 (17 μg/mL) cancer cell line respectively while the furanocembranoid SC1-8 (0.01 %) (***28***) best suppressed inflammation in LPS induced RAW 264.7 cell and zebra fish embryo at 10 μg/ml concentration. As such, the adaptation of marine invertebrate in the marine environment caters to great potential as an important source of lead pharmaceutical metabolites.

ABSTRAK

KEPELBAGAIAN STRUKTUR DAN CIRI-CIRI BIOLOGI METABOLIT KEDUA DARIPADA LINTAH LAUT (APLYSIA DACTYLOMELA) DAN BATU KARANG LEMBUT ALCYONACEAN.

Borneo Utara, yang terletak dalam Kawasan Segitiga Terumbu Karang (KSTK), merupakan biodiversiti mega dunia, mempunyai sumber hidupan marin yang kaya. Ekosistem marin merupakan satu gabungan interaksi antara persekitaran dengan organisma marin yang membawa kepada hungan mangsa pemangsa, persaingan untuk ruang atau habitat, perlindungan daripada penyakit atau jangkitan dan mengakibatkan tekanan terhadap invertebrata yang lambat atau tidak bergerak. Oleh itu, invertebrata marin membentuk metabolit sekunder untuk beradaptasi dan melindungi diri secara kimia. Pemajuan dalam penemuan ubat-ubatan telah mendedahkan potensi metabolit sekunder marin sebagai berpotensi sebagai ubat-ubatan farmaseutikal. Kajian ini memberi tumpuan kepada dua invertebrata; lintah laut Aplysia dactylomela *Rang dan karang lembut Alcyonacean (*Lobophytum pauciflorum, Scleronephthea corymbosa *dan* Sinularia flexibilis*) untuk menyiasat kepelbagaian struktur metabolit sekunder dan potensi biologinya. Populasi A. dactylomela dari Pulau Dinawan menghasilkan 10 sebatian, Pulau Sulug menghasilkan 9 sebatian dan Pulau Mantanani menghasilkan 12 sebatian, yang terdiri daripada lima sebatian baru dengan dua rangka kimia baru. Sebatian yang dipencil kebanyakannya mengandungi unsur halogen dan terdiri daripada struktur acetogenin, charmigrane, cuparane, syndrean dan bromoindole. Tiga spesis karang lembut Alcyonacean dianalisis mengandungi 22 metabolit sekunder;* Lobophytum pauciflorum *menghasilkan 6 sebatian,* Scleronephthya corymbosa *menghasilkan 6 sebatian dan* Sinularia flexibilis *menghasilkan 10 sebatian yang terdiri daripada furanocembranoids, strerol jenis pregnane dan cembrane diterpene. Metabolit sekunder dari karang lembut menghasilkan lima sebatian baru. Penentuan struktur sebatian dilakukan melalui bacaan 1D-NMR (proton dan karbon), 2D-NMR (HSQC, COSY, HMBC dan NOESY), HRMS dan FTIR. Sesquiterpena (+)-elatol (***16***) dan 2,3,5,6 - tetrabromoindole (***22***) merencatkan pertumbuhan Escherichia coli (HP0408), Vibrio cholera (HP0908) pada kepekatan MIC 5 $\mu g/mL$ dan 10 $\mu g/mL$ masing-masing. Syndrean 12-acetoxypalisadin B (0.02 %) (***13***) dan sterol pregnane P3N-4 (0.01 %) (***32***) menunjukkan sitotoksisiti arah sel kanser HL60 dan B16F10 manakala furanocembranoid SC1-8 (***28***) merencatkan keradangan dalam sel RAW 264.7 dan embrio ikan zebra yang dirawat dengan LPS. Oleh itu, melalui kajian ini adalah jelas bahawa penyesuaian invertebrata marin dalam persekitaran ekstrim menyebabkan organism ini mengandungi kepentingan sebagai sumber bagi metabolit berpotensi farmaseutikal.*

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LIST OF ABBREVATIONS AND SYMBOLS

MeOH	Methanol
H_2O	Water
dH_2O	Distilled water
Na_2SO_4	Sodium sulphate anhydrous
EtOAc	Ethyl acetate
N_2	Nitrogen
Hex	Hexane
$CHCl_3$	Chloroform
CMW	Chloroform:Methanol:Water
R_f	Mobility relative to front
CC	Column Chromatography
HPLC	High Performance Liquid Chromatography
PTLC	Preparative Thin Layer Chromatography
HRMS	High Resolution Mass spectrometry
TLC	Thin Layer Chromatography
UV	Ultra violet
^{1}H-NMR	Proton Nuclear Magnetic Resonance
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
2D-NMR	Two dimensional Nuclear Magnetic Resonance
HMBC	Heteronuclear multiband correlation
HSQC	Heteronuclear single-quantum coherence
NOESY	Nuclear Overhauser enhancement spectroscopy
EIMS	Electron Ionization Mass Spectrometry