

**INVESTIGATION ON ANTI-INFLAMMATORY
ACTIVITIES AND MECHANISMS OF ACTION
OF BACTERIA ASSOCIATED WITH MARINE
SPONGE *HALICLONA AMBOINENSIS***

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Over the past few decades, inflammation has been recognized as a major risk factor for various human diseases. They have three major group of drugs used in threaten of inflammatory diseases, such as corticosteroids, non steroidal anti-inflammatory drugs (NSAID) and disease modifying anti rheumatoid drugs (DMARDs). Unfortunately, these drugs may have too many side effects in the body. Therefore, an alternative drug from the nature especially from marine natural product was investigated in this study to confirm their potency as anti-inflammatory agent. Study on marine sponge *Haliclona amboinensis*-associated bacteria as anti-inflammatory potential agents was done by inhibiting of inducible nitric oxide synthase (iNOS), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and phospholipase A2 (sPLA₂) enzymes. Result revealed that all samples were inhibited COX-1, COX-2 and sPLA₂ enzymes activities compare to arginine as a control. The highest activity in inhibiting of COX-1 enzyme activity was yielded by fraction Fr 1at concentration

of 2.5 µg/ml with percentage of inhibition is $116.88 \pm 4.698\%$ followed by MeOH extract $116.64 \pm 0.235\%$. The highest activity in inhibiting of COX-1 enzyme activity was yielded by fraction F1C4 at concentration of 2.5 µg/ml with percentage of inhibition is $116.45 \pm 3.289\%$ followed by fraction F1C2 ($107.36 \pm 0.235\%$). Furthermore, all samples were inhibited sPLA₂ enzyme activity, with the highest inhibition was obtained by fraction F1C2 ($114.81 \pm 0.940\%$), followed by fraction F1C4 ($105.55 \pm 0.282\%$), fraction Fr2 ($88.89 \pm 0.507\%$), Fr 1 ($85.18 \pm 0.235\%$), fraction F1C3 ($81.48 \pm 0.003\%$) and methanol crude ($70.26 \pm 1.175\%$). This study provide evidence to show that methanol actives extract and fractions of *H. amboinensis*-associated bacteria have anti-inflammatory activity with proposed mechanism of action by inhibiting iNOS, COX-1, COX-2, and sPLA₂ enzymes activity. Chemical constituents from alkaloids and terpenoids group could be contributed in this anti-inflammatory activity since phytochemical analysis result showed that alkaloids and terpenoids were present in selected methanol extract. Moreover, bacteria isolated from *H. amboinensis*-associated bacteria which produce anti-inflammatory agents were identified as *Halomonas* sp.

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**KAJIAN ANTI-RADANG DAN MEKANISMA TINDAKAN DARI
BAKTERIA YANG BERASOSIASI DENGAN SPAN LAUT
*HALICLONA AMBOINENSIS***

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Sejak beberapa dekad yang lalu, keradangan telah diiktiraf sebagai faktor risiko utama kepada pelbagai jenis penyakit manusia. Terdapat tiga kumpulan utama ubat-ubatan yang digunakan dalam mengubati penyakit radang seperti kortikosteroid, ubat anti-radang bukan steroidal (NSAID) dan ubat-ubatan ubahsuai untuk penyakit anti rheumatoid (DMARDs). Walau bagaimanapun, ubat-ubatan ini mungkin memberi banyak kesan sampingan kepada manusia. Oleh itu, ubat alternatif daripada alam semula jadi terutamanya daripada produk semula jadi marin telah dikaji dalam kajian ini untuk mengesahkan potensi mereka sebagai agen anti-radang. Kajian ke atas bakteria yang berhubungan dengan span laut *Haliclona amboinensis* sebagai potensi agen anti-radang telah dilakukan dengan menghalang daripada *inducible nitric oxide synthase* (iNOS), *cyclooxygenase-1* (COX-1), *cyclooxygenase-2* (COX-2), dan *phospholipase A2* (sPLA2) enzim. Hasil kajian mendapati kesemua sampel telah menghalang COX-1, COX-2 dan sPLA2 enzim aktiviti dibandingkan dengan arginina sebagai kontrol. Aktiviti tertinggi

dalam menghalang COX-1 aktiviti enzim telah dihasilkan oleh pecahan Fr 1 pada kepekatan 2.5 µg / ml dengan peratusan perencatan adalah $116.88 \pm 4.698\%$ diikuti dengan pecahan MeOH extract $114.64 \pm 0.235\%$. Aktiviti tertinggi dalam menghalang COX-2 aktiviti enzim telah dihasilkan oleh pecahan F1C4 pada kepekatan 2.5 µg / ml dengan peratusan perencatan adalah $116.45 \pm 3.289\%$ diikuti dengan pecahan F1C2 ($107.36 \pm 0.235\%$). Tambahan pula, semua sampel telah menghalang aktiviti enzim sPLA2, dengan perencatan yang tertinggi telah diperoleh oleh pecahan F1C2 ($114.81 \pm 0.940\%$), diikuti dengan pecahan F1C4 ($105.55 \pm 0.282\%$), pecahan FR2 ($88.89 \pm 0.507\%$), Fr 1 ($85.18 \pm 0.235\%$), F1C3 pecahan ($81.48 \pm 0.003\%$) dan ekstrak metanol ($70.26 \pm 1.175\%$). Kajian ini membuktikan bahawa ekstrak aktif metanol dan pecahan *H. amboinensis*-bersekutu bakteria mempunyai aktiviti anti-radang dengan mekanisme cadangan tindakan dengan menghalang iNOS, COX-1, COX-2, dan sPLA2 enzim aktiviti. Kandungan kimia dari kumpulan alkaloid dan terpenoid boleh menyumbang dalam aktiviti anti-radang kerana hasil analisis fitokimia menunjukkan alkaloid dan terpenoid hadir dalam ekstrak metanol dipilih. Selain itu, bakteria diasingkan daripada *H. amboinensis*-bersekutu bakteria yang menghasilkan agen anti-radang telah dikenal pasti sebagai *Halomonas* sp.