

HORNED STARFISH (*Protoreaster nodosus*)
EXTRACT FOR DIABETIC WOUND IN WHITE
RATS

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**HORNED STARFISH (*Protoreaster nodosus*) EXTRACT FOR DIABETIC WOUND
IN WHITE RATS**

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HORNED STARFISH, (*Protoreaster nodosus*) EXTRACT FOR DIABETIC WOUND IN WHITE RATS

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Institute : Institute of Marine Biotechnology

Sea cucumber's ability as a medicine especially in wound healing is well known and may contribute to overexploitation in future. Hence, an alternative possessing equal ability is needed. Based on previous study and the ability to regenerate, starfish; an asteroidean under the phylum Echinodermata has been chosen. Horned starfish, *Protoreaster nodosus* was dried, extracted, fractioned and desalted for topical application on diabetic wound healing study. Prior to the application, bioassay tests were conducted comprising of the antibacterial activity, antioxidant assay and cytotoxicity test at different concentrations. Pathogenic and wound associated bacteria were tested against the aqueous methanol extract (0.08 mg/ml, 0.16 mg/ml, 0.31 mg/ml, 0.63 mg/ml, 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the antioxidant activity. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was applied for the cytotoxicity of the extract with concentrations of 0.94 µg/ml, 1.88 µg/ml, 3.75 µg/ml, 7.5 µg/ml, 15 µg/ml,

30 µg/ml and 60 µg/ml. Fatty acid of the extract was determined as one of the nutritional values. In wound healing study, 36 diabetes induced rats were subjected to two full dermal thickness wound punch. Application of the extract was done topically, twice a day for a period of 14 days and another 14 days without treatment for delay toxicity study. Body weight, blood glucose level, leukocyte count, gross wound observation and collagen deposition was analyzed through histology. Toxicity effect was analyzed by evaluating the leukocyte count and morphology changes of the kidney and liver via histology. From the bioassay tests, aqueous methanol extract had antibacterial activity towards *Staphylococcus aureus*, high antioxidant activity (0.23 mg/ml, 90.34% of inhibition) and was not toxic towards L6 cell. Fatty acids composed of PUFA, EFA and carotenoid fatty acid. As for the wound healing study, significant different in wound contraction was observed in extract group ($P < 0.05$). Collagen deposition was significant ($P < 0.05$) when compared to unwounded skin. No toxic effects were observed in the kidney and liver of the rats. These findings revealed the potential of starfish as a wound healing promoter with antibacterial activity, antioxidant property and high fatty acid composition.

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EKSTRAK TAPAK SULAIMAN BERTANDUK (*Protoreaster nodosus*) UNTUK
LUKA DIABETIS KE ATAS TIKUS PUTIH

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Keupayaan gamat sebagai ubat terutamanya sebagai ubat luka boleh mengakibatkan ia dieksploitasi tanpa kawalan pada masa hadapan. Bagi menangani masalah ini, alternatif yang mempunyai keupayaan yang sama diperlukan. Berdasarkan kajian terdahulu dan kebolehan untuk tumbuh semula, tapak sulaiman; asteroidia di bawah filum ekinodermata telah dipilih. Tapak sulaiman bertanduk, *Protoreaster nodosus* telah dikeringkan, diekstrak, dibahagikan dan dinyah garam untuk tujuan sapuan ke atas luka diabetik. Sebelum aplikasi, beberapa ujian bioesei terdiri daripada aktiviti antibakteria, ujian antioksidasi dan ujian toksik ke atas sel telah dijalankan dalam beberapa siri kepekatan. Bakteria merbahaya dan bakteria di kawasan luka telah digunakan untuk menguji kesan ekstrak akueus metanol (0.08 mg/ml, 0.16 mg/ml, 0.31 mg/ml, 0.63 mg/ml, 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml) manakala 2,2-diphenyl-1-picrylhydrazyl (DPPH) telah digunakan untuk menentukan aktiviti antioksidasi. Untuk

ujian toksik terhadap sel, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) telah digunakan ke atas ekstrak berkepekatan 0.94 $\mu\text{g/ml}$, 1.88 $\mu\text{g/ml}$, 3.75 $\mu\text{g/ml}$, 7.5 $\mu\text{g/ml}$, 15 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$ and 60 $\mu\text{g/ml}$. Salah satu nilai nutrisi ekstrak yang ditentukan ialah asid lemak. 36 ekor tikus telah digunakan dalam kajian penyembuhan luka dimana setiap tikus mengalami dua luka dermis penuh. Aplikasi ekstrak dilakukan secara sapuan topikal sebanyak dua kali sehari bagi tempoh 14 hari manakala 14 hari berikutnya tanpa aplikasi bagi tujuan kajian toksik yang tertangguh. Berat badan, kandungan gula dalam darah, kiraan leukosit, pemerhatian luka secara kasar dan pembentukan kolagen telah dianalisa secara histologi. Kesan toksik telah dianalisa melalui kiraan leukosit dan perubahan morfologi pada ginjal dan hati tikus. Daripada ujian bioesei, ekstrak akueus metanol mempunyai antibakteria aktiviti terhadap *Staphylococcus aureus*, tinggi dengan aktiviti antioksidasi (0.23 mg/ml, 90.34% of perencatan) dan tidak mempunyai kesan toksik terhadap sel L6. Asid lemak terdiri daripada PUFA, EFA dan asid lemak karotenoid. Bagi kajian penyembuhan luka, pengecutan luka adalah ketara di dalam kumpulan ekstrak ($P < 0.05$) apabila dibandingkan dengan kumpulan kawalan. Pembentukan kolagen pula adalah ketara ($P < 0.05$) apabila dibandingkan dengan kulit tanpa luka. Tiada kesan toksik ditemui pada ginjal dan hati tikus. Penemuan-penemuan ini mendedahkan potensi tapak sulaiman dalam membantu proses penyembuhan luka dengan antibakteria aktiviti, keupayaan antioksidasi dan kandungan asid lemak yang tinggi.